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Plant responses to sublethal concentrations of 2,4-D

Bert T. Swanson
Iowa State University

PLANT RESPONSES TO SUBLETHAL CONCENTRATIONS OF 2,4-D

by

Bert Theodore Swanson

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INTRODUCTION

Weeds are among the greatest contributors to food production costs. There is an estimated annual loss of 5 billion dollars due to weeds in the Agriculture industry (74) and an additional $2.5 billion is spent annually to hold these losses to a minimum (56). No comparable figure is available for the Horticulture industry, but this loss is believed to equal the total loss attributed to insect and disease injury.

The wide spread use of the systemic herbicide 2,4-D (2,4-dichlorophenoxy acetic acid), has provided a major contribution in the production of an efficient and reliable weed control program. Its selectivity at herbicidal concentrations is well known and the importance of its use in agriculture has been firmly established. However, serious injury to susceptible non-treated plants has resulted from spray drift, vapor drift and residues in the soil and this injury often occurs at very sublethal concentrations. Freed (29) has reported injury of horticultural crops after the agriculture use of a volatile form of 2,4-D as far as 40 miles away. It is apparent therefore, that immediate local restrictions on the use of 2,4-D will not alleviate the problem and total abandonment of 2,4-D use seems unlikely due to its economical herbicide properties for specific extensively cultivated crops. The use of low volatile forms of 2,4-D is, however, of considerable benefit in reducing the amount of vapor drift and consequently, injury to other plants.

Symptoms of 2,4-D injury may be slight upon first exposure to sublethal concentrations but tend to become more severe with time indicating a possible accumulation of the parent compound, its derivatives, or a

metabolite of the compound in the plant tissue. Voluminous research has contributed much to a possible mode of action but conflicting results, and the great variability of possible existing conditions make it difficult to postulate a distinct mechanism of action. Previous workers (13, 29) that have shown injury due to a combination of spray drift and vapor drift have experienced difficulty in determining the concentration of 2,4-D in the ambient atmosphere at which this injury occurs.

To determine an approximate concentration of spray drift and vapor drift at which plants might be affected and what these effects would be, a study was conducted using the ambient atmosphere as well as a controlled atmosphere in which a known concentration could be maintained for a given period of time. The objectives of the study were: 1) to investigate the presence, amount and accumulation of 2,4-D in tomato plants from an exposure to the amount of 2,4-D in the ambient atmosphere and from an exposure to a calibrated amount added at a given rate; 2) to determine internal concentrations of 2,4-D in the plant in relation to the amount of injury found; 3) to determine the effects of sublethal concentrations of 2,4-D on the degree of parthenocarpy in tomatoes; 4) to determine the effect on yield and size of fruit of tomatoes and strawberries; 5) to determine the effect of 2,4-D on the yield of green beans; and 6) to relate visual symptoms obtained on the treated plant foliage to the amount of 2,4-D to which they were exposed and to the amount of 2,4-D contained within the tissue.

REVIEW OF LITERATURE

Recent comprehensive reviews of the literature on herbicides, in which 2,4-D is considered, are numerous (3, 6, 62) as well as specific reviews emphasizing the mode of action of 2,4-D (16, 38, 78).

History of 2,4-D

Perhaps one of the greatest advances in solving the problems arising from weeds in crops came with the synthesis of 2,4-D acid by Robert Pokorny in 1941 (64). In 1942, Zimmerman and Hitchcock (93) determined the hormone-like properties of this white, nonhygroscopic crystalline material and described it as a plant growth regulator. Marth and Mitchell (54), in 1944, established its selectivity by removing plantain, dandelion and other broad-leaved weeds from bluegrass lawns. In the same year, Hamner and Tukey (37) successfully used 2,4-D to control weeds in certain field crops. In 1959, herbicides, of which 2,4-D is a major type, were applied to an estimated 53 million acres of the nation's croplands, and in 1962 over 70 million acres were treated (56).

Forms and Uses of 2,4-D

Chemical manipulation of 2,4-D formulations has been done in an attempt to produce cheap, convenient, nonvolatile, noncorrosive products having satisfactory shelf life, easy handling properties not subject to decomposition or change in form from environmental conditions, while still maintaining all of the selective and toxic properties of the parent form.

The acid of 2,4-D is only slightly soluble in water and if applied in the form of a spray it must be rendered soluble as one of the various salts

of the acid that is readily soluble in water and usually as effective as the acid itself (21). The acid molecule is small and mobile yet not highly volatile. Its chief disadvantage seems to be its low water solubility, however, this does give it a buffering action to regulate both penetration and contact toxicity. If the hydrogen in the carboxyl group is replaced by an organic group such as methyl (CH_3), ethyl (C_2H_5) or butyl (C_4H_9) group, an ester, or organic salt, is formed. Large scale application of the methyl, ethyl, butyl and amyl esters have given results comparable to the application of the acid and of the ammonium salts (21). Davis (21) indicates that the esters of 2,4-D are much more effective than the water soluble salts but cannot be used as safely because of their volatility and the risk of vapor drifting to sensitive vegetation.

Mullison (59) has shown that the alkanolamine salt and the sodium salt of 2,4-D were non-volatile as determined by responses of tomatoes, beans, and cotton plants. However, aliphatic (1 to 5 carbon) esters of 2,4-D were sufficiently volatile to cause decided plant injury. Short exposures of 15 minutes at 100 degrees F. or 30 minutes at 75 degrees F. to the vapor of the methyl esters of 2,4-D were sufficient to cause a plant response. Mullison's experiments indicate that as the number of carbon atoms in the aliphatic portion of the 2,4-D ester is increased, the volitility is decreased.

On the basis of a rather sensitive biological test, the acid, sodium salt, triethanolamine salt and amide forms of 2,4-D were nonvolatile. All esters (methyl, ethyl, butyl, isobutyl, amyl, isoamyl, isopropyl, alkyl, phenyl, beta-chloroethyl, and n-octyl) volatilized and produced growth effects on the test plants. Methyl, ethyl, butyl, and isopropyl appeared

to be more volatile than the others.

The 2,4-D acid and its related compounds, with their outstanding and unusual herbicidal properties, have become some of the leading synthetic plant hormones. By selective action they are highly toxic to most broad-leaved plants and relatively nontoxic to monocotyledonous plants. Other selective uses include prevention of preharvest fruit drop and the production of seedless fruit (7, 9, 33, 47, 48).

Effective as it is when used judiciously, 2,4-D may give disappointing results when used by an inexperienced person. There is no universal procedure that will control all weeds for all sections of the country; nor is there an overall prescription of formulation which will be equally effective against all weed pests under all conditions. Marth and Mitchell (54) found 2,4-D acid to be effective as a differential herbicide when applied as an aqueous spray in concentrations of from 250-1,000 ppm or more. It was possible to obtain 95% control of dandelion and narrow-leaved plantain by a single spray application of a solution containing 1,000 ppm of 2,4-D acid or with two applications at 500 ppm concentration. Davis (21) reported that 200 gallons per acre of a 0.05% to 0.15% solution completely eradicated most common turf weeds with 2 to 4 weeks. This was true regardless of the 2,4-D formulation except for wild onion and knotweed. When water is used as a carrier for 2,4-D, Gleason (31) found that the spray was more effective than in either diesel oil, motor oil or an oil-water combination. He attributed this to greater injury of the leaves, thereby disrupting translocation of 2,4-D into the plant.

Surfactants or wetting agents have been used to enhance the absorption and penetration of 2,4-D into the plant. Foy (27) defines a surfactant as

any substance which is capable of altering the energy relationship at surfaces or interfaces. He reported they may facilitate and accentuate the emulsifying, dispersing, spreading, wetting, solubilizing, and/or other surface modifying properties of herbicidal formulations to bring about enhancement of penetration and herbicidal action. Jansen, Gestner and Shaw, and McWhorter, as cited by Jansen (41), have demonstrated that toxicity of herbicides could be greatly enhanced by some surfactants, unaffected by others, or significantly suppressed by a third group. Jansen (41) describes the reaction as a herbicidal-surfactant-species interaction. Other investigators have also reported a variety of responses with the use of surfactants and 2,4-D (52, 32, 67, 70).

A major problem in the use of 2,4-D has been the effect of spray drift on sensitive non-target plants. Woodford and Evans (85) have indicated that the drift hazard with 2,4-D is a serious one because of the high activity of the spray and dusts against many crop plants and also the application of 2,4-D as concentrated solutions in low volumes per acre. Spray drift is likely to be most troublesome in areas where there is a mixture of horticultural and non-horticultural crops. According to Woodford and Evans (85) drift may occur in three ways: spray drift, which results from smaller droplets in the spray being carried away from the target by wind or convection currents; vapor drift, which occurs when the vapor from a volatile formulation is carried away from the target area during and after spraying and; blow off, which is movement by high winds of dried spray particles or herbicide-impregnated soil away from the area originally treated. Vapor drift occurs mostly in hot weather and can take place even if the air is apparently still.

King and Kramer (45) studied the effects of the vapors of certain polyethylene glycol esters, and the butyl esters of 2,4-D and 2,4-5-T on unsprayed tomato and cotton plants enclosed in chambers with plants sprayed with these esters. No marked toxicity to the unsprayed plants was observed with the polyethylene esters but severe epinasty resulting in death occurred with the butyl esters. Dry cucumber seeds were not injured by storage with the polyethylene glycol esters for seven days at 32, 70 or 90 degrees F., however, when stored with the butyl esters of 2,4-D they were appreciably injured at all three temperatures.

Damage resulting from spray and vapor drift can be minimized or prevented by the use of low volatile forms of 2,4-D, by use of specially designed spray equipment, and also by caution on the part of those applying the herbicide. The acid, a salt or a low volatile ester should be used in preference to the highly volatile esters in areas where other plants sensitive to 2,4-D are growing. Ennis and Williamson (25) found that small droplets (below 0.1 millimeters diameter) of all herbicidal formulations were markedly more inhibitory to all test plants than larger droplets (over 0.3 millimeters diameter). Young and Fisher (89) observed that larger droplets are more effective in reducing the drift hazard. Regardless of equipment or formulation used, wind conditions at time of spraying, spraying pressure, and rate of application are important factors relating to the control of spray and vapor drift. Caution and precision appear to be the most satisfactory solution to these spray drift and vapor drift problems.

Plant Responses to 2,4-D

Growth

Wort (86) has stated that the effects of applied herbicides on the metabolism and composition of a living plant are complex, seldom static, and may change quickly and to a remarkable degree. The numerous factors which determine the metabolic and composition status of the plant are those associated with 1) the herbicide; 2) the plant; 3) the environment.

The herbicide 2,4-D, is a growth promoting substance similar to the naturally occurring indol auxins. According to Van Overbeek (76) a difference between 2,4-D and the indol auxins is that 2,4-D is far more active and it persists in the plant for a longer period of time.

Gorter and Van Der Zweep (33) reported the formation of tubiform or cuplike organs and that connations are common after application of auxin herbicides. A connation occurs when primordia or two or more organs fuse at an early stage of development and then grow together. Numerous atypical tropistic responses have been observed after treatment with 2,4-D (38). Brown (11) reported that within one hour, bean seedlings sprayed with a 1,000 ppm concentration showed epinastic response and stem bending, which became more severe during the next two or three days and after five days the plants were permanently wilted. Leaf growth and expansion were markedly inhibited in both partially expanded leaves and those contained in terminal buds, even when sprayed with a concentration as low as 25 ppm. Swanson (73) found that yeast growth was not stimulated by any concentration of 2,4-D and that the inhibition of growth was related to the degree of saturation of the active growth sites by 2,4-D. Rogers (65) observed that stalk brittleness of two varieties of corn was apparent when exposed

to 2,4-D at the six to eleven leaf stage. Tukey et al. (75) with 1,000 ppm of 2,4-D acid applied to vigorously growing plants observed that pollen grains were plasmolyzed and disorganized, flowers were arrested in development and chlorophyl formation was checked. Bradbury and Ennis (8) showed that both soil and leaf treatment of kidney bean plants with 2,4-D caused partial stomatal closure. Kiermayer (44) showed that the formation of intercellular spaces is prevented by treatment with 2,4-D. Cell division is greatly increased in all cambial zones and phloem regions (75) and also in the corpus and lead primordia as early as twenty-four hours after treatment (39).

Liao and Hamilton (49) report evidence detected by autoradiographic techniques that exogenously supplied 2,4-D can be localized in both the cytoplasm and the nuclei of root-tip cells. The cytoplasmic labeling decreased with time and was not observed after 120 hours, however, nuclei labeling was found at all time intervals.

Eames (24) has shown that it is the tissues between the cortex and primary xylem of bean seedlings which are primarily involved in responding to 2,4-D. In the primary phloem, the parenchyma cells proliferate freely, disrupting the phloem strands within which they lie and soon crush the companion cells and smaller sieve tubes. He states that the destruction of the phloem is certainly a contributing factor in the killing of the bean seedling with 2,4-D. Swanson (72) stated that meristematic tissue and those tissues capable of reverting to a meristematic condition are most readily affected by 2,4-D. The derivatives of such tissues remain meristematic for considerable periods, and if differentiation occurs it is never in an orderly fashion.

It is not surprising that a compound is effective when applied as a vapor or a solution since its molecular constitution does not necessarily change when the physical condition is changed from a solid to a gaseous state. Zimmerman et al. (95) studied 29 compounds which were physiologically active as plant growth substances when applied in solutions and found them to be active also when applied as vapors to several species including tomato, corn, and garden peas. Zimmerman et al. (94) reported growth responses resulting from 2,4-D contamination of insecticide formulations, storage areas of 2,4-D, application equipment, 2,4-D spray drift during spraying operations in nearby areas or air pollution caused by 2,4-D vapors. Amounts of 2,4-D as low as 0.0001% in agricultural formulations were determined by growth response induced on tomato and cotton plants. Zimmerman (91) has observed that malformation of sensitive species can be induced at will and have been observed on phlox, roses, garden beans, wistaria, oak, cotton, and tomatoes around factories manufacturing 2,4-D. Davis (21) has reported that plant susceptibility to sublethal exposure of 2,4-D is markedly influenced by the condition of the plant and also by environmental factors. Since most of the injury symptoms are expressed by growth responses, the plant must be developing new leaves to show the injury. He showed that sublethal quantities of 2,4-D applied to growing points produces in time, stunting, distortion, vein clearing and mottling. Of the plants Davis observed in the field, he found the grape to be the most sensitive to 2,4-D. Tumorization is a characteristic response of many plants to rather low concentrations of 2,4-D (52). Loomis (52) also reports that although stimulation of cell division by 2,4-D, particularly in meristematic regions, is a common response, it appears to

be more characteristic of minimal or sublethal doses on resistant plants than an invariable accompaniment of 2,4-D treatment. Guzman (36) exposed tomato plants in a confined atmosphere and in the field to both spray drift and volatility in separate experiments. Symptom severity at the end of a twelve hour period showed a straight line relationship between wind and temperature. The tomatoes treated at low wind and low temperature showed practically no symptoms of 2,4-D while those treated at high wind and high temperatures showed twisting and bending of the stem. It was apparent that although drift definitely causes damage to susceptible plants when using the butoxy ethanol low volatile ester of 2,4-D, volatility of the material was also important. Zimmerman et al. (95) sprayed test plants with a deVilbiss atomizer using concentrations ranging from 0.0001% to 10%. Vapors from isopropyl and the butyl ester of 2,4-D induced the same type of formative effects as those induced when 2,4-D acid or salt was applied by other methods. The degree of severity of the effects is proportional to the length of time of exposure. Cole (13) reported that in 1962, 2,4-D injury symptoms appeared by July 6th due to a build up of the concentrations of 2,4-D in the atmosphere and by August 6th it had declined sufficiently to allow recovery of the injured plants. He demonstrated that the effect of this ambient 2,4-D was to increase the total growth of wood but decrease the total photosynthetic area of the plant. Not only are growing plants subject to injury from vapors but Mullison and Hummer (60) have shown that seeds should not be stored where they will be exposed to the vapor of volatile esters of 2,4-D. Aliphatic (1 - 5 carbon) esters of 2,4-D are sufficiently volatile to adversely effect the germination of numerous field crop and vegetable seeds exposed to such vapors.

Maturity

According to Derschied (23), the stage of growth is the most important single factor affecting 2,4-D injury. He reports that barley and oats are most susceptible at the five leaf stage but are tolerant at the milk stage. Rossman and Staniforth (67) also showed that inbred lines of corn were most susceptible at the six to eight leaf stage while application made at tasseling time and ten days after pollination produced no visible effect. Williams et al. (82) reported that while tomatoes were ruined commercially at relatively low concentrations of 2,4-D, they were not easily killed upon reaching a height of ten to twelve inches. Perennial weeds which possess storage organs, such as the dandelion with its tap root and the wild onion with its bulb are most easily killed when the carbohydrate reserves in the storage organs are low and those in the leaves high (21). Some perennial weeds can be killed with 2,4-D applied at any time of the year even when they are dormant. He also reported that the time of day at which 2,4-D is applied does not seem to influence the results so far as eradication of weeds or the growth of grass is concerned. Zielinski and Garren (90) have demonstrated that application of ten ppm of 2,4-D to Montomorency cherry trees monthly and semimonthly resulted in a delay of four to six weeks in fruit maturity, a larger number of fruits per fascicle and a retention of fruit on the tree during the fall and winter. Cole (13) suggested the age of the plant has a definite effect on the amount of 2,4-D required for production of injury symptoms. He stated that once leaves reach maturity there is seldom any visible evidence of 2,4-D injury until the cumulative exposure reaches a point that causes necrosis of the leaf, even though the younger tissues of the plant will show symptoms at a much lower concentra-

tion. Cole (13) also noted a predominant difference in the ripening process as related to the exposure to 2,4-D.

Yield

Derscheid (22) found that early application of 2,4-D to barley, appears to injure vegetative primordia which results in fewer tillers, fewer spikes, and lower yield. The degree of yield reduction depends on the rate of growth. If growth is rapid, differentiation is slow and yield reduction is slight but if the growth rate is slow differentiation is rapid and large yield reduction results. Application at the time of anthesis causes large yield reductions. Derscheid (22) attributes this to poor pollination and, therefore, the production of fewer seeds per spike. Rossman and Staniforth (67) observed serious reduction in seed yield in four inbred lines of corn when sprayed at the six and eight leaf stages. Rogers (65) reported similar reductions in yield. Guzman (36) observed no significant reduction in yield of five week old tomato plants upon exposure to spray drift or vapor drift. Holt (39) observed yield reduction of Andrew and Cherokee varieties of oats after treatment of one pound acid equivalent per acre of n-butyl ester of 2,4-D applied from nine to thirty-eight days after planting. Cole (13) showed that yield of grapes was decreased slightly as the distance to a volatile source of 2,4-D (butyl ester) was decreased. Wort (88) found that the deformative effects and reductions in yield caused by application of weed controlling concentrations of 2,4-D to Marquis spring wheat and other cereals could be prevented by the inclusion of ferrous ions in the spray.

Parthenocarpic development

As set forth by Gorter and Van Der Zweep (33) the normal sequence of events preceding fruit set is: pollination, germination of the pollen grain, growth of the pollen tube into the style, release and fusion of pollen tube nuclei with the nuclei in the embryo sac, and finally growth of the embryo. Growth of the fruit starts after the growth of the embryo begins. If no pollination or fertilization occurs, apomictic embryos originate from one or more unfertilized cells in the embryo sac or neighboring tissues. Bonner and Galston (7) report that the growth of fruit depends intimately on auxin. The source of this auxin in general is in the developing seeds of the fruit, however, fruits may develop in some plant species in the absence of seed formation. Unpollinated ovaries of tomato, petunia and other species will develop into fruits if an active auxin source is available and applied to the style (7). Gorter and Van Der Zweep (33) report that the source of this auxin appears to be the tissue of the fruit itself. Bonner and Galston (7) have found that the artificial application of auxin can replace the need for pollination in fruit development. They found IAA, IBA, a- and b-napththoxyacitic acid and 2,4-D to be effective. Sastry and Muir (68) found 1×10^{-5} M gibberillic acid and 1×10^{-2} M IAA to induce parthenocarpy in tomatoes. Fruit development through influences other than pollination, such as application of an auxin to the unpollinated ovary, results in seedless fruits due to absence of pollination and fertilization which prohibits the normal development of seeds.

Leopold (47) describes three types of parthenocarpy: 1) fruit development without any pollination: tomatoes, peppers, pumpkins, and

cucumbers; 2) fruit development stimulated by pollination but proceeding to full development even without the pollen tubes ever reaching the ovule and effecting syngamy: orchids, peas and triploid plants; 3) seedlessness as a result of the abortion of the embryo before the fruit reaches maturity: cherries, peaches, and grapes. Gustafson (35) was the first to report the use of well known synthetic growth substances to induce parthenocarpic development. He reported the production of seedless tomatoes, peppers, eggplant, cucumber, and several others. Osborne and Went (63) were able to induce parthenocarpy in tomatoes with low temperature and high light intensities which are the conditions in which pollination is poor. Britten (9) with corn, and Gustafson (34) with tomatoes showed parthenocarpic development after treatment with B-napthoxyacetic acid. The latter reported that the seedless fruits were larger than the seeded fruits and also that fruit set was somewhat greater than with open pollination.

Leopold and Frances (48) have shown that the capacity for fruit set in tomatoes is dependent upon temperature. This dependency has been explained on the basis of excess growth of the style, reduced pollen viability and inhibition of pollen tube growth as well as the inherent temperature-sensitivity in the tomato ovary itself. The optimum range for fruit set is approximately 18 - 22 degrees C. (48).

Photosynthesis and respiration

Several investigators report a reduction in the rate of photosynthesis upon treatment with 2,4-D (2, 16, 38, 86). Since seedlings treated with 2,4-D die without even starting photosynthesis, Crafts (16) reports its effects on photosynthesis are a secondary effect which Wort (86) suggested

to be closure of the stomates. Wort (86) also reported that 2,4-D at lethal levels has an adverse effect on the rate of photosynthesis, but at low concentrations applied at the correct time could result in an increase in the net photosynthetic rate.

In contrast to photosynthesis the respiration rate of plants is significantly increased as a result of treatment with 2,4-D (11, 38, 40, 51, 52). Loomis (51) reported a maximum of 92% increase of respiration in dandelions treated with 480 ppm 2,4-D. Loomis (52) has postulated that increased respiration in tissues treated with 2,4-D is due to: 1) increased substrate, either by mobilization or by stimulating digestion of reserve carbohydrates; 2) increased cell division and protoplasm synthesis in treated tissues so that more respiring substance is present in the same volume of tissue; or 3) increased permeability of other toxicity effects which permit a more rapid action of enzymes on a substrate. Probably all three of these effects are present at different times and in varying proportions.

Carbohydrate and nitrogen metabolism

Plants treated with 2,4-D tend to show a rapid depletion of sugars and starch in a wide variety of plants (2, 19, 38, 46, 52, 57, 75, 86). Klingman and Ahlgren (46) propose that reserve carbohydrates are rapidly hydrolyzed to reducing sugars which are in turn oxidized through an active catabolic system, resulting in a total loss of dry weight. Mitchell and Brown (57) found that readily available carbohydrates (sugars, starch, and dextrin) were essentially depleted within three weeks in vigorous and relatively dormant plants. Carbohydrate reserves (starch and dextrin)

were also rapidly depleted in the flower buds and roots of annual morning glory. Hilton et al. (38) found increased total carbohydrates in dwarf bean. They also reported increased starch but decreased sugar in potato stems, and disappearance of starch from the cortex and pith of tomato shoots. Loomis (52) reported that treatment with 2,4-D results in the digestion of starch or other polysaccarides and in the temporary accumulation of sugars. He also indicated that plants do not die of starvation, as dying plants still contain considerable quantities of available carbohydrates.

Klingman and Ahlgren (46) found an increase in the percent total nitrogen in 2,4-D treated plants which they contributed to a more rapid utilization of the carbohydrate fraction than the nitrogen fraction. The number of milligrams of total nitrogen per plant was, however, reduced in the treated plants. Freiberg and Clark (30) observed changes in distribution of different forms of nitrogen of treated and control plants as well as different responses of the plants at high and low nitrogen levels. Visible responses to 2,4-D appeared sooner at the high nitrogen than the low nitrogen levels. Although no difference appeared between high and low nitrogen 24 hours after treatment, the percent dry matter of the leaves was significantly less than the controls. Several days later the leaves of treated plants wilted and showed a much higher percent dry matter. Treated plants showed a decrease in the percent of protein nitrogen in the leaves and an increase of soluble organic nitrogen. A slight increase in total nitrogen was noted one day after treatment, however, after four days the treated plants contained significantly less total nitrogen than the controls. Wolf et al. (84) also noted different plant responses at different

nitrogen levels. Treated plants receiving high nitrogen were dead fourteen days after treatment; at this same time the medium nitrogen plants showed severe chlorosis of leaves and stems and splitting of stems but the low nitrogen plants showed only mild chlorosis. Hilton et al. (38) reported that only consistent observation on nitrogen metabolism of 2,4-D treated plants was a decrease in amino and amide nitrogen. Total and protein nitrogen was variable and dependent on species.

Water and mineral uptake

Brown (11) has reported that the total amount of water absorbed and transpired by 2,4-D treated plants during the five days immediately following treatment was 34 percent less than comparable untreated plants. The rate of accumulation of water in the leaves of sprayed plants was depressed, while in the stem tissue it was accelerated. However, on an over-all basis, the treated plants had a higher percent moisture than the untreated ones. Wort (86) indicates that effects dependent on a changed water content may be the earliest visible symptoms of the action of 2,4-D on plants and reports excess turgidity often results from the application of 2,4-D. He also points out that 2,4-D either depresses or has little effect on the intake of some fourteen ions by plants and in very few instances is the uptake increased. An increase of phosphorus uptake by plants has been shown by some investigators (26, 88) while it has also been shown to be decreased (81). Rhodes, as cited by Wort (86), observed no appreciable change in phosphorous uptake in 2,4-D treated tomato tops. Wildon et al. (81) has shown that the tops of treated plants contained a lesser percent of potassium, sodium and phosphorous and a greater percent of boron and iron.

Roots accumulated more calcium and copper. Little difference was shown in accumulation of calcium, copper, magnesium and zinc in the tops, nor in accumulations of boron, potassium, magensium, manganese, and phosphorous in the roots.

From the numerous and variable effects of 2,4-D it is apparent that the final results are determined not only by the general nature of the herbicide but also its chemical form, by the concentration used, the pH, the carrier, the wetting agent, the method of application, and the size of droplets or dust particles applied. The species of plant, the part of the plant to which the chemical is applied, the plant's age, vigor and past history all play a part in determining the response to 2,4-D. Add to all these factors the environmental conditions at the time of application and during the period the herbicide is active within the plant, and it becomes quite apparent that the condition of the treated living plant at a given instance is indeed determined by many variables.

Absorption of 2,4-D by Plants

According to Loomis (52) the protoplasm of the epidermal cells of the typical plant tissue is covered by four protective layers: the cytoplasmic membrane, the epidermal cell wall, the cuticular layer and commonly, by a greater or lesser amount of extruded waxy deposit over the surface of the cuticle. Waxes are hydrophobic and resistant to wetting with pure aqueous sprays and therefore interfere or prevent contact of a spray droplet with the leaf surface. Foy (27) indicates that waxy leaf surfaces are normally readily wet only by aqueous sprays containing a suitable surfactant. He cautions, however, that enhanced wetting is not always synonymous with

enhanced penetration. Cutin is described by Foy (27) as a semilipoidal oxydative polymer of long chain fatty acids and alcohols. It may contain appreciable quantities of polymerized carboxylic acid in which case, having many polar groups, such cutins may absorb water and swell. This hydration spreads apart the wax components and tends to increase the permeability of the cuticle to polar molecules and promotes and absorption of water-soluble herbicides. The pectic layer and the cellulose layer are hydrophilic or polar and are therefore not considered an important obstacle to the penetration of aqueous sprays. Foy (27) further reports that in general there exists a gradient from low polarity at the exterior of the cuticle to relatively high polarity in the layers bordering the epidermal cell wall. Lipophilic waxes predominate toward the outside with the outer layers containing only wax and semilipoidal, semipolar cutin. Hydrophilic substances, cellulose and pectins, are in predominance in the inner region.

The polarity of herbicide molecules determines their solubility in the carrier solutions, the cuticle, the cell wall and cell membrane. The less polar the molecules, the more lipid-soluble they are. Undissociated solutes are relatively non-polar and therefore are oil-like and penetrate lipoid barriers more readily.

Crafts (16) reports four possible fates of an applied herbicide with respect to penetration: 1) it may remain on the outer leaf surface and dry down to the crystalline form or it may remain in liquid form depending on the constituents of the formulations; 2) it may penetrate into the cuticle and then pass into the aqueous phase of the epidermal cell walls and migrate via the anticlinal walls to the vascular system; 3) it may penetrate the cuticle and then part into the aqueous phase of the

epidermal cell walls and migrate via the anticlinal walls to the vascular system; 4) it may follow the latter route into the leaf and then be absorbed into the symplast and thence move to the phloem and out of the leaf in the assimilation stream. In this latter process part of the active ingredient may be absorbed into vacuoles of cells along its route. The chlorophenoxy compounds are an example of the fourth process in that they penetrate the cuticle, migrate across the mesophyll and move into the phloem.

Both surfaces of leaves appear to function in the absorption of 2,4-D. Foy (27) reported the lower epidermis more penetrable than the upper and that not all areas of the leaf are especially permeable. He classifies penetration as either stomatal or cuticular, although even after stomatal penetration, a lipoidal barrier still exists. Open stomates constitute a port of entry for spray solutions (16), however, the roll of stomatal uptake under field conditions is conflicting. Weaver and DeRose (77) report stomates to be unimportant as a port of entry except perhaps upon exposure to the more volatile compounds or to an aerosol. Foy (27) has postulated that penetration may generally involve the following:

> For aqueous solutions of inorganic salts, acids, bases, and polar organic compounds, entry is through cracks, punctures or areas of leaves not completely covered by waxy lamellae and then following a polar (aqueous) route by the hydrated cutin and/or the hydrophilic pectin and cellulose portions of the wall. For oils or polar solutes, absorption takes place directly through the waxy portion of the cuticle via an apolar (lipoid) route. For substances exhibiting both polar and apolar properties which tend to render compatible in the spray mix-plant surface complex, transport is via a combined aqueous and lipoid route through the cuticle proper as well as throughout imperfections.

There is evidence that 2,4-D is more readily absorbed as an undissociated molecule (4, 52). Investigators (4, 12, 52) also report that Q_{10} data

indicate the absorption process is not simple diffusion but rather some type of chemical or enzymatic reaction. Rice, as cited by Leopold (47), observed a rapid but brief uptake of 2,4-D at 32 degrees C. and a slow but prolonged uptake at lower temperatures.

Weintraub et al. (80) showed that absorption of 2,4-D by leaves increases to a peak during growth, drops markedly with maturity, then remains constant until a further drop to a very low level as senile change becomes prominent. Leopold (47) also found two phases of 2,4-D uptake by leaves: a rapid initial uptake which was followed by a slower, steady uptake.

Crafts (16) summarized the absorption of 2,4-D in plants by suggesting that both the lipoid and aqueous routes are available under most conditions and the relative importance of either depends upon the condition of water in the plant (stress or saturation), the nature of the molecules applied (lipoid or water soluble), and the formulation of the compound.

Translocation of 2,4-D in Plants

Translocation of herbicides involves the movement of toxins within living tissue and probably sensitive tissues whose functioning depend upon the maintenance of more or less normal metabolism. Leopold (47) reports movement may occur: 1) in the xylem along with the transpiration stream, 2) through the phloem or other cells such as ray parenchyma, 3) through the cell walls, or 4) through the intercellular spaces. Several investigators have reported that translocation of 2,4-D is closely associated with translocation of carbohydrates (4, 5, 15, 21, 58, 66). In multi-leaved bean plants, absorption and translocation were greatest from the lower unifoliate leaves, less from successively higher leaves and that no 2,4-D was

transported from young leaves that were still importing food from more mature parts of the plants. Barrier (4) has shown that in soybeans a supply of sucrose is necessary for translocation of 2,4-D and that any 2,4-D absorbed is held in the leaves until carbohydrates become available. These workers indicate that 2,4-D moves upward through the xylem when absorbed by the roots and up and down in the plant through the phloem at a rate dependent on temperature. The rates of translocation of 2,4-D show the temperature coefficient of a chemical reaction (4, 5). This may be as fast as 9 meters per hour in the zylem and 10 to 100 centimeters per hour in the phloem (47) but showing some variability depending on the concentration in which higher levels increase absorption but decrease translocation, and also depending on the use of a surfactant which may enhance both absorption and translocation. Translocation across parenchyma tissue of the leaf or root is usually at rates of millimeters or at best a few centimeters per hour (17). Crafts (17) reports that flowers and young fruits are extremely active sinks and invariably accumulate a high concentration of 2,4-D when it is applied to plants at this stage of development. Accumulation of 2,4-D to toxic concentrations is also possible in other portions of the symplast (16, 17, 29). Crafts (17) reports that 2,4-D tends to accumulate in active parenchyma cells and in this manner is limited in translocation. Slife et al. (69) indicates that 2,4-D is moved primarily in the phloem and is accumulated in the regions of rapid growth and that very little is moved into the fully developed leaves.

Since there is much evidence that transport of 2,4-D is from regions of synthesis of foods to regions of their utilization, movement from early leaves of seedlings may be predominently into roots. From later leaves

movement may be to both roots and shoot tips and from upper mature leaves it may be predominently into growing shoots, flowers and fruits. Studies with labeled herbicide molecules have shown that 2,4-D, during migration across the mesophyll and during movement in the phloem, is subject to accumulation within living cells. Crafts (16) postulates that such accumulation takes place in the vacuoles of parenchema cells and represents a type of storage. He maintains that 2,4-D is subject to accumulation in vacuoles along its entire route of transport and that direct contact killing of leaves by 2,4-D is not a result of phloem crushing but it may well be a manifestation of accumulation by active parenchyma.

Metabolism and Mode of Action of 2,4-D

It has been postulated that in many instances, death from 2,4-D is not directly due to some specific internal cause but rather is brought about by the invasion of saprophytic and parasitic micro-organisms, favored by the physiological and morphological abnormalities of the deranged plant (34). According to Weintraub (78) the 2,4-D molecules may be thought of as having four sites of specificity: the ring, the ether oxygen, the methylene group and the carboxyl group. Although the esters, amines, and salts are most commonly used in the field, evidence shows that conversion to the free acid is necessary for the expression of herbicidal activity (38). Crosby (18) reports that free 2,4-D appears to be the major chlorine containing ether soluble constituent of the bean plant four days after treatment and that this constituent of 2,4-D is indeed the parent herbicide itself. This metabolic fate of phenoxyocetic acid includes: 1) physical or chemical conjugation with cellular constituents, 2) degradation

of the aliphatic acid side chains of the molecule and 3) ring hydroxylation. Hilton et al. (38) further shows that susceptible and resistant plants degrade the acetic acid side chain of 2,4-D and release the carboxyl methylene carbons as CO_2. A logarithmic disappearance of 2,4-D was observed in bean plants with about 70 percent still recoverable after five days but only about five percent recoverable after 25 days (47).

From previous evidence presented it is apparent that 2,4-D also tends to be accumulated in certain portions of the plant. In an experiment with ironweed, Linscott and McCarty (50) found that the accumulation in various parts was related to the stage of growth of the plant at the time of treatment. When field plants were treated in the active vegetative growth stage, greatest accumulation was observed in newly expanding leaves, leaf petioles, auxiliary buds and above ground portions of the stem.

Although 2,4-D remains one of the most widely used herbicides in the United States, and although a great amount of excellent research has been accomplished, its precise mechanism of action continues to remain a subject of research. 2,4-D is said to be an auxin similar to IAA in that both compounds influence the increase in size of cells responsible for polarized growth and growth curvatures, initiation of roots, activation of the cambium, stimulation of callus, correlative inhibition of lateral buds, stimulation of fruit development and ripening, and abscission of organs. In these cases, 2,4-D behaves like an auxin; however, it is also a powerful herbicide in which respect IAA is relatively impotent. Weintraub (78) reports that there is a correlation between the formative response to 2,4-D and the lowered endogenous auxin content of the plant. 2,4-D disrupts the orderly process of leaf development by causing, in the meristematic layers,

an early cessation of the anticlinal divisions while permitting the cells to divide periclinally. This results in a leaf blade which is much narrower and thicker than the normal leaf. There is evidence (13, 79) that 2,4-D can persist during the dormant season and induce morphological abnormalities when growth resumes the following season. Weintraub et al. (79) reports that such responses could be due to a reaction of the 2,4-D with cells that are formed long after the treatment. Frans (28) has postulated a herbicide (H) will combine reversibly with some mechanism or reactive site (M) within the plant to form a herbicide-mechanism complex (HM). This complex is then thought to be transferred irreversibly into products which ultimately give rise to inhibition of growth. This concept is expressed by the following equation:

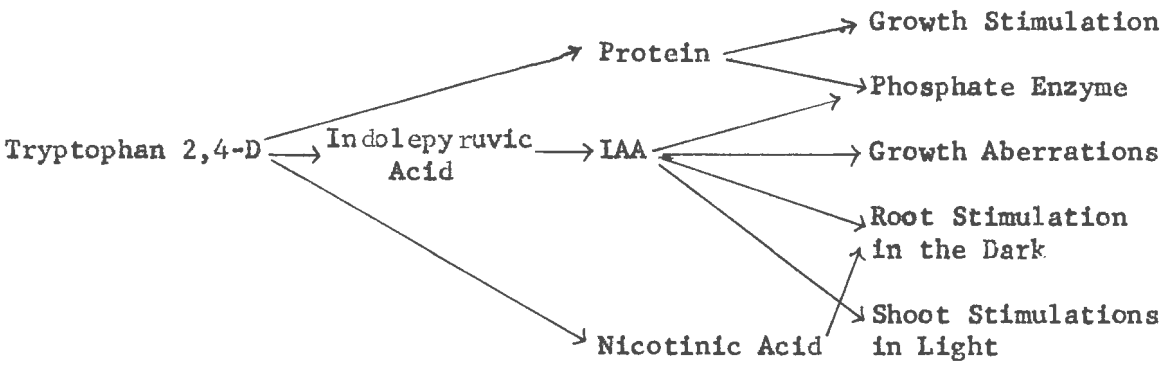
$$H \;\&\; M \underset{K_2}{\overset{K_1}{\rightleftarrows}} HM \xrightarrow{K_3} \text{Inhibition of Growth}$$

in which K_1, K_2 and K_3 are rate constants of the two reactions. He postulated that 2,4-D probably combines with the mechanism or site necessary to auxin-induced growth until all these available sites are saturated and that inhibition does not occur until these sites are completely filled and the herbicide "spills over" onto secondary sites lending to inhibition or combines with compounds necessary to the normal functioning of growth. Some investigators suggest that a two point attachment (52) to the susceptible growth site is involved while others suggest a three point attachment (78).

Since minute quantities of 2,4-D produce marked changes in the chemical composition, such as reduction of carbohydrates and an accumulation of nitrogen, it is indicated that an enzyme system might be involved. Neely

et al. (61) shows that 1000 ppm 2,4-D considerably lowers the activity of both alpha and beta amylase in the stems of bean plants but had no effect in the leaves. Hilton et al. (38) reported that 2,4-D causes an increase in pectin methylesterase activity. Wort (87) has suggested that 2,4-D effects the phosphorylative portion of the respiratory sequence through the inactivation or combination of an enzyme. Many of the studies on the effect of 2,4-D on plant enzyme are contradictory and some investigators feel the response on the enzyme systems are produced indirectly.

Crafts (16) has suggested the following scheme based on a reaction with tryptophan to explain the wide array of responses to 2,4-D by plants:



If 2,4-D should be able to regulate the transformations shown above, increases in protein might explain the growth stimulation of cotton shoots, grape tendrils and seedlings observed in the light and root growth in the dark. Increases in IAA might inhibit growth and bring about the morphological aberrations commonly observed on 2,4-D treated plants. In proper combinations, IAA and nicotinic acid might stimulate root initiation and growth. A favorable combination of protein and IAA might result in increased phosphatase which in turn would account for depletion of stored food. Such a combination of effects might help to explain the stimulation

found from low dosage applications of 2,4-D; the translocations into and accumulation in meristems of 2,4-D resulting in injury and death from intermediate dosages, and the immediate contact killing and restricted translocation resulting from heavy applications of the readily absorbed esters.

MATERIALS AND METHODS

Greenhouses and Outdoor Plots

In an effort to obtain and maintain an atmosphere free from ambient 2,4-D, six quonset type, aluminum framed, plastic greenhouses (Treatments 1, 2 and 3) were constructed (Figures 1 and 2). Each house was 21.75 feet wide, 32.25 feet long and 9.5 feet high. Two plots of similar area were also maintained without plastic coverings (Treatment 4). In the summer of 1966, four mil plastic was used and found to be inadequate to endure the entire growing season. In 1967, six mil, ultra violet light resistant plastic was used which lasted satisfactorily throughout the entire season. The plastic was removed during the winter and the houses were recovered in late April in 1966 and in early May in 1967. Each greenhouse was equipped with an exhaust vent on the north end (Figure 1) and with two intake fans on the south end (Figure 2). The fans pulled air through wet excelsior pads to cool the houses and to maintain a positive pressure within the structures. Through the use of smoke, it was found that the distribution pattern of air within the houses provided satisfactory dispersal to all portions of the structure.

In addition to the cooling pads, the air in four of the structures (Treatments 1 and 2) was also pulled through Barnebey - Cheney activated charcoal filters which would absorb any 2,4-D from the ambient atmosphere and therefore provide a "2,4-D free" atmosphere to these four houses (Figure 3). Houses five and six (Treatment 3) had ambient air flowing through them and the outside plots, areas seven and eight (Treatment 4) were maintained in ambient atmosphere. Furnace filters were placed over the

Figure 1. Exhaust vents on north end of greenhouses

Figure 2. Ventilation and filter systems on south end of greenhouses

Figure 3. Charcoal filters under aluminum shelters on the ventilation systems of Treatment 1

charcoal filters in houses 1 through 4 and over the cooling pads in houses 5 and 6 to help prevent dust accumulation in the filters and pads. These were changed approximately every two weeks. Operation of the fans was controlled manually; however, operation of the pumps that pumped the water into the pads was controlled by thermostats located within the houses.

A daily record was maintained on all equipment failures and plastic breakage. In 1966, several pump failures made it necessary to open the units for repair. To monitor environmental conditions, thermographs were placed outside and in all houses in instrument shelters constructed approximately three feet high in the houses and five feet high outside. Two thermometers were placed in the outside plots and eleven thermometers were distributed in each house including one directly in front of each fan and one at the exhaust vent. The remaining eight were spaced throughout the houses in aluminum reflectors at eight foot intervals in the two rows ten feet apart and four feet from the ends of the houses. Thermometer and psychrometer readings were taken from one to three p.m. once each week during the 1966 growing season. In 1967 these readings were taken every two weeks. Continuous soil temperature was recorded in house number one in 1966 and in house number two in 1967. A summary of temperature and humidity conditions for the 1966 and 1967 growing seasons are as shown in Figures 4 through 7.

Plant Material

On April 28 and 29, 1966, sixty Ozark Beauty strawberries (Fragaria sp. L.), six Fallred raspberries (Rubus strigosus L.), and six Concord grape (Vitis labrusca L.) were planted in all eight plots. On May 20

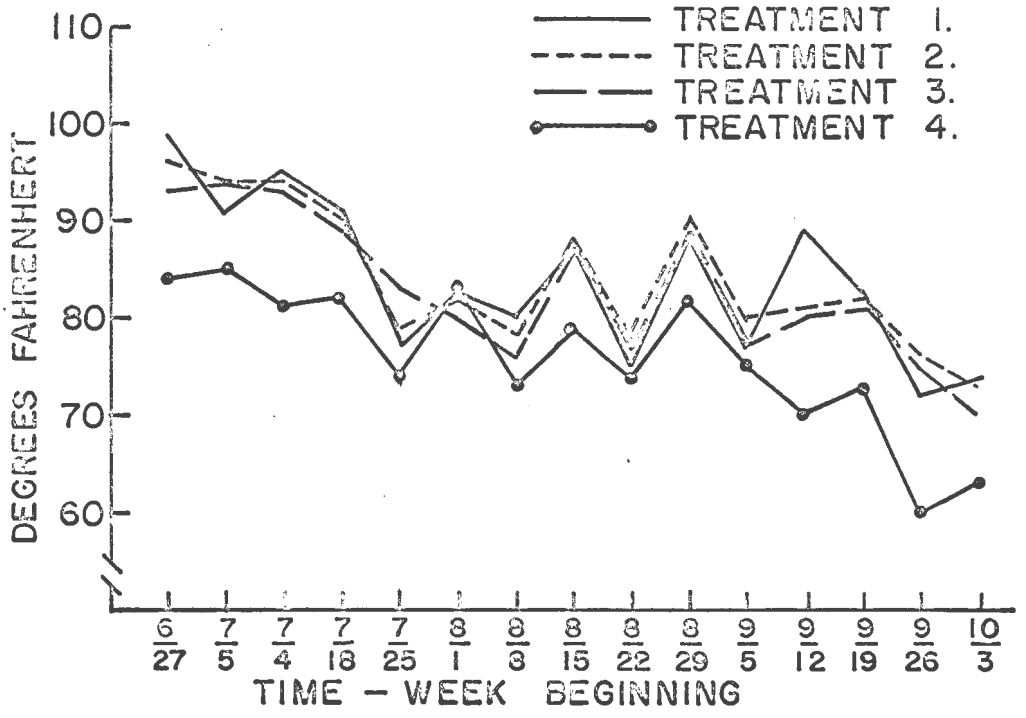


Figure 4. 1966 treatment temperature

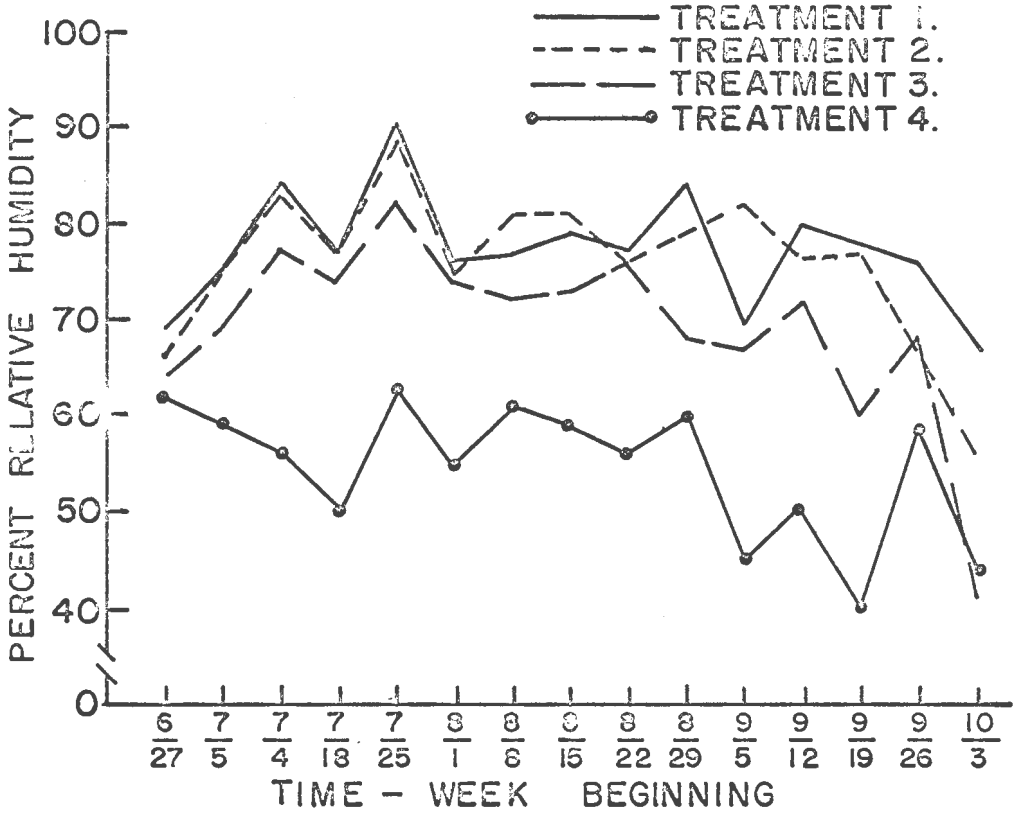


Figure 5. 1966 treatment relative humidity

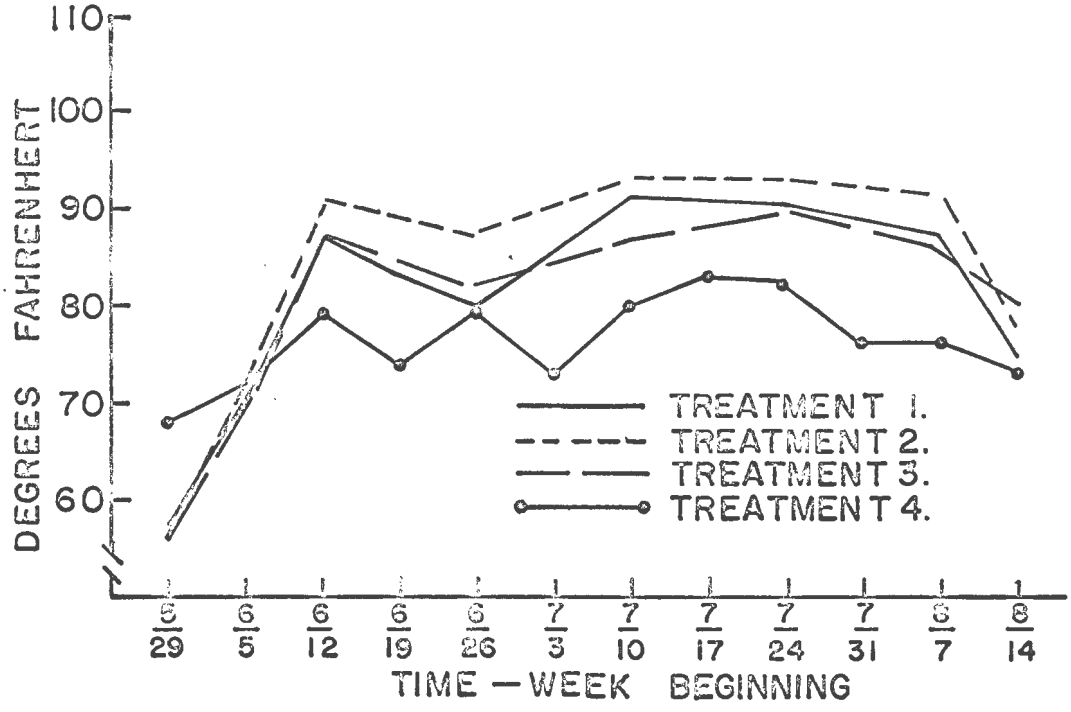


Figure 6. 1967 treatment temperature

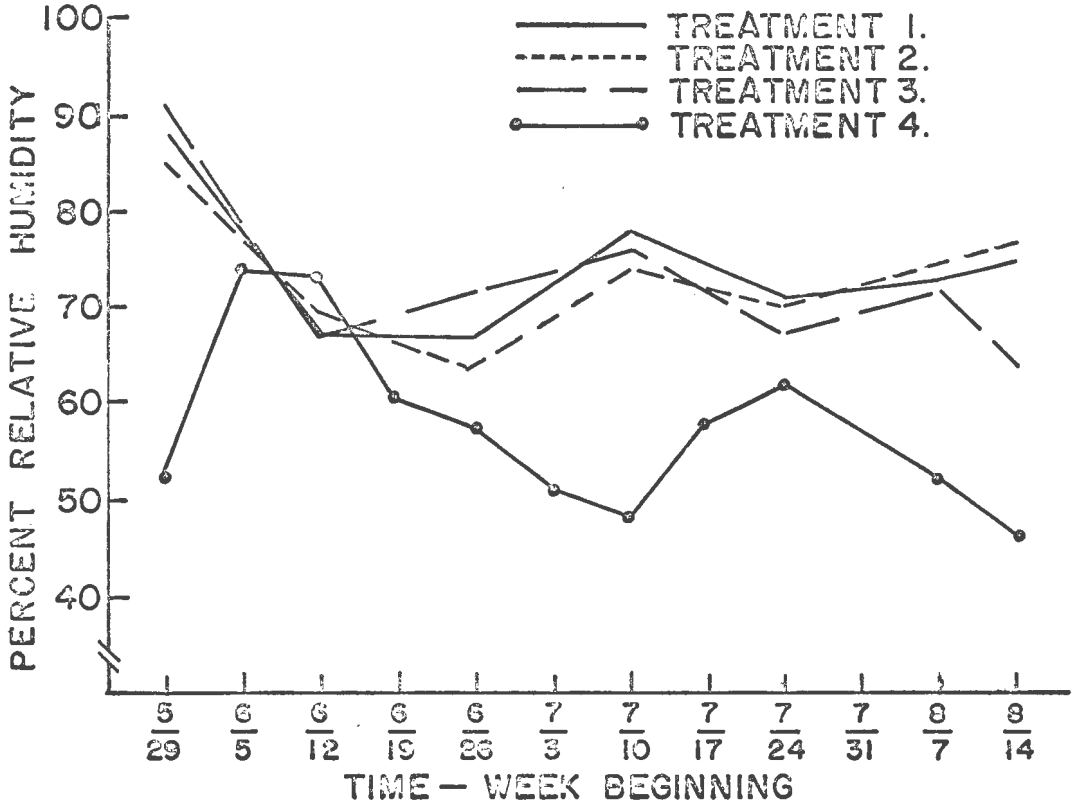


Figure 7. 1967 treatment relative humidity

three varieties of tomatoes (Lycopersicum esculentum M.) were seeded directly into the soil. The varieties included Campbell 1327, a prominent canning variety, Avalanche and Manapal. Studies in southwestern Iowa by Weigle[1] indicate that Avalanche is extremely sensitive to volatile 2,4-D and work in the Ames area indicates that Manapal is somewhat tolerant to volatile 2,4-D. After planting, all plots received an application of 10-10-10 commercial fertilizer, which was then followed by an application of a one to two inch layer of crushed corn cob mulch.

In 1967, the plant material was the same with the exception of the tomatoes in which only the Campbell 1327 variety was planted. The plant material also included the addition of greenbeans (Phaseolus vulgaris S.) variety tendergreen. Tomatoes, sown in pots in a heated greenhouse on February 28, 1967, were transplanted to the plastic houses on May 24, 1967. The beans were seeded on May 25, 1967. The planting diagrams are as described in Figure 8 for 1966 and Figure 9 for 1967. The numbers indicate a particular plant number which is then prefixed by the plot number (number one through eight) for complete identification of each plant.

Treatment Procedure

In a quantitative study of 2,4-D esters in the air, Adams et al. (1) found an average concentration of the butyl ester 2,4-D in the ambient atmosphere to be 0.07-0.12 $\mu g/m^3$ in an eastern Washington area. The maximum concentration found was 2.2$\mu g/m^3$. They found the average concentration to exist eighty percent of the time in which they sampled.

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[1]Weigle, J. L., Iowa State University, Ames, Iowa. Data from field study on tomatoes. Private communication. 1966.

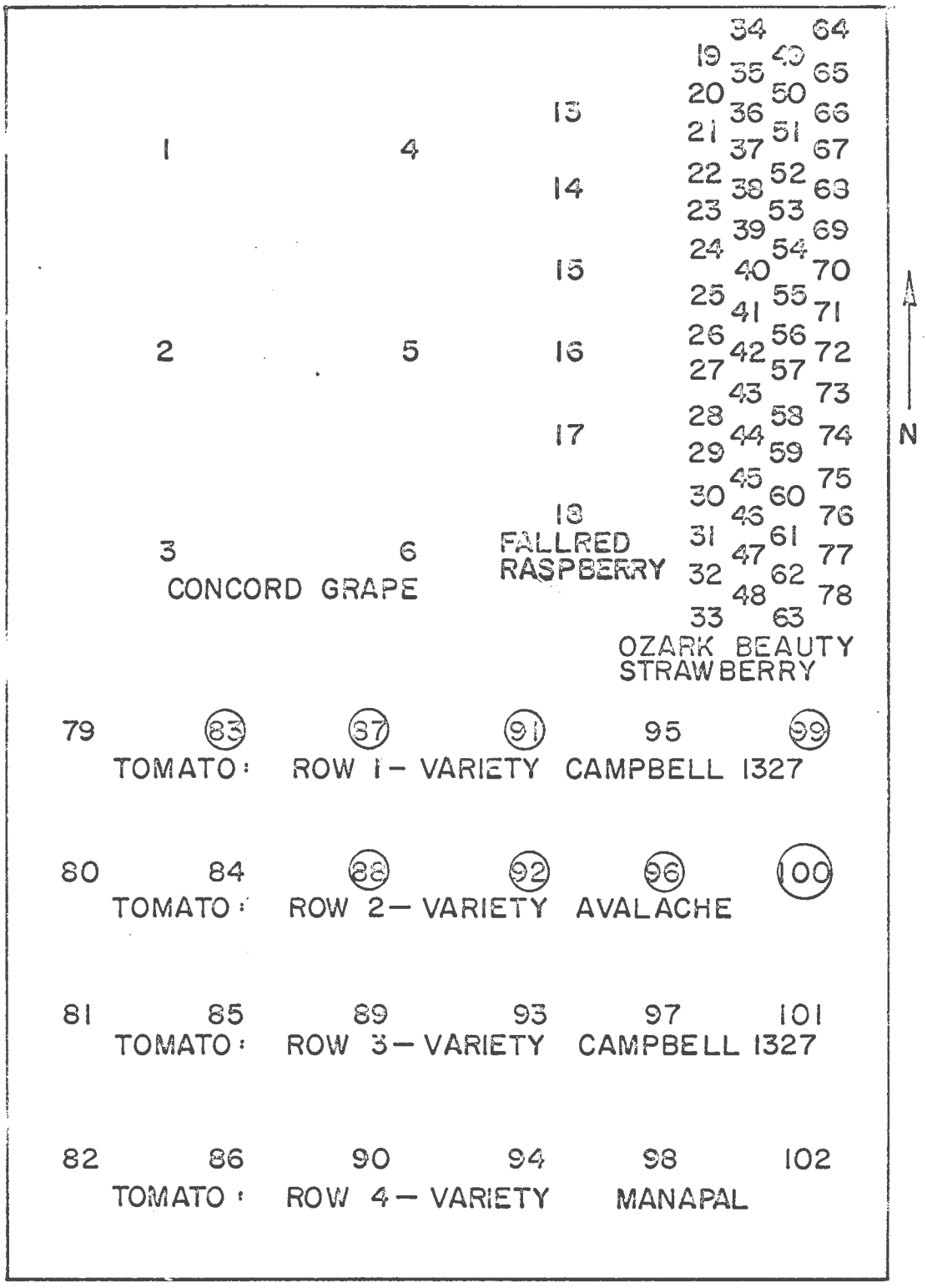

Figure 8. 1966 planting diagram

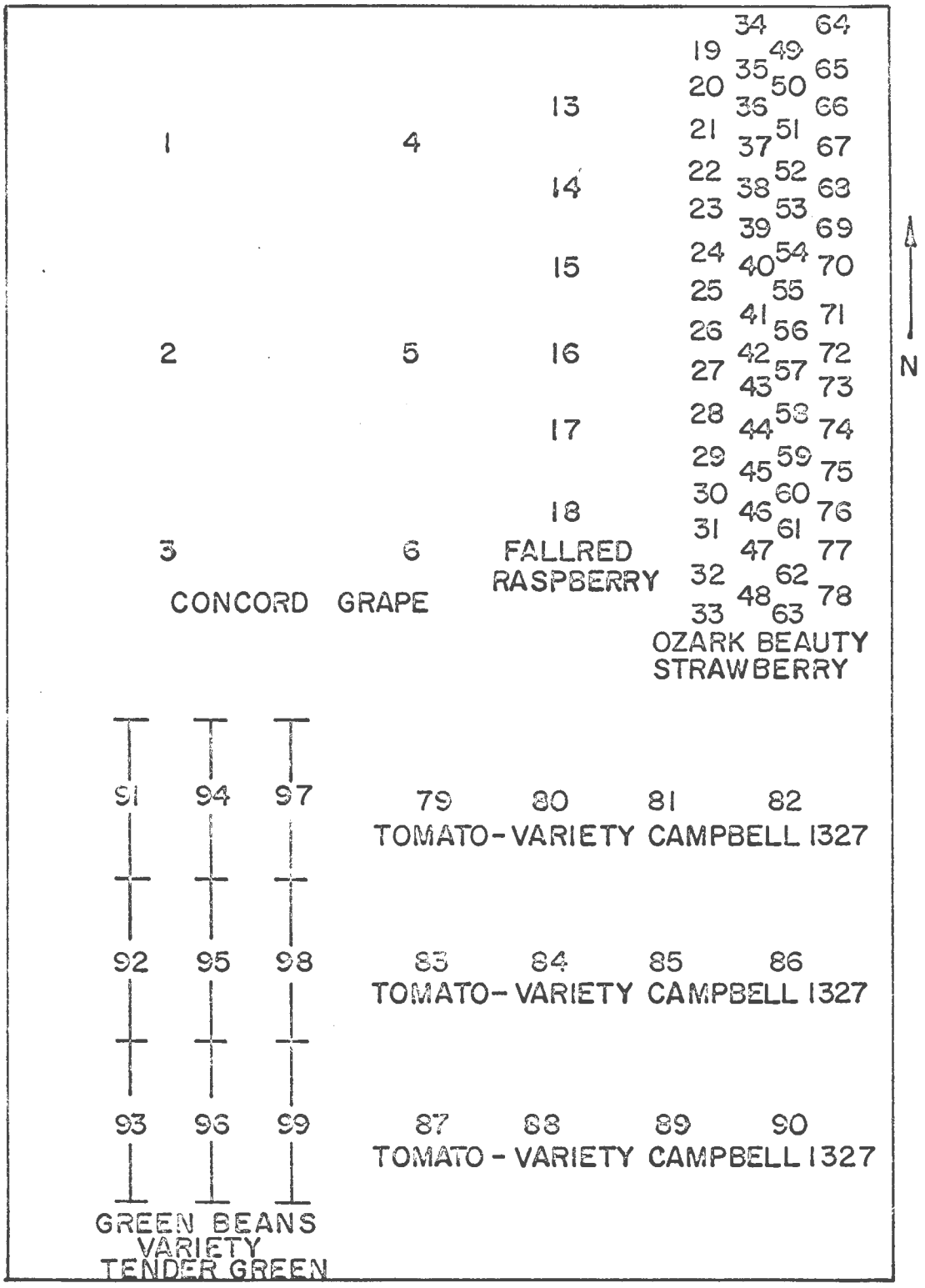

Figure 9. 1967 planting diagram

Using this information, 2,4-D exposures were made with a one part per billion solution of the butyl ester in water, maintained for six hours in Plot 1 in 1966 and in Plots 1 and 2 in 1967 (Treatment 1). This is computed on a weight to weight basis using the weight of air at 30°C. On a weight-volume basis it equals 1.18 $\mu g/m^3$ which is slightly higher than the average found by Adams et al. (1) and slightly lower than their maximum dosages found in the ambient atmosphere. Flow measurements indicated that the flow rate through Plots 1 and 2 was equal to an 80 percent air exchange per minute in 1966 and a 65 percent air exchange per minute in 1967. This decrease is due to an addition of charcoal to the filters prior to the 1967 season. The amount of actual 2,4-D added in 1966, 48.1 mg per house, was adjusted to 39.1 mg in 1967 to account for the decrease in flow rate.

The butyl ester of 2,4-D was dissolved in a petroleum solvent at a ratio of one to ten to increase its solubility in water. This solution was then diluted in enough water to allow atomization to extend for six hours. The solution was atomized through the use of a deVilbiss atomizer embedded in a rubber stopper and placed in a 4000 milliliter flask (Figure 10). Air pressure for atomization was supplied in 1966 from a cylinder of water pumped compressed nitrogen gas, and in 1967 by a Gast Manufacturing Corporation air compressor. The flasks were then placed in front of the air intake fans of the houses to be treated and a fine mist blown into the fans under five to seven p.s.i. of air pressure (Figure 11). The air from the fans then helped to volatilize the mist and force it back throughout the house.

Due to two electrical power failures on the first and fourth of July

Figure 10. Apparatus used for the atomization of 2,4-D

Figure 11. Atomization system used in releasing 2,4-D in Treatment 1

1966, which resulted in air temperature in excess of 125° within the houses, treatment was delayed until the heat damage was no longer prominent on the vegetation. The first treatment therefore was made in Plot 1 on July 25, 1966, from 0700 to 1300 and then continued once each week until September 26, 1966, for a total of ten treatments. Plot 2 was treated four times in the same manner except that only the petroleum solvent was used. No abnormalities were observed after this time so treatment of Plot 2 was discontinued. In 1967 the first treatment was made on July 3 from 0600-1200 in Plots 1 and 2 and continued each week until August 7, 1967.

Determination of Weights, Counts and Parthenocarpic Development

Tomatoes in 1966 and strawberries and greenbeans in 1967 were weighed according to row and plot immediately after each harvest. The tomatoes and strawberries were also counted according to each row and plot. All eight plots (four treatments) were used in determination of this data.

Up to 25 tomatoes from each row in each plot were checked for parthenocarpic development. Each tomato was quartered and the number of seeds in the quarter used as an estimation of the number of seeds in the whole fruit. Since a statistical correlation of 0.91 was obtained for this relationship it was considered adequately workable. The average number of seeds present in the group of tomatoes checked was then used in the statistical analyses of treatments, harvests and rows.

Plant Sampling

Tomato plants were sampled to determine the content of 2,4-D in the foliage. Rows 1 and 2, varieties Campbell and Avalanche, were selected for sampling as they were the farthest from the source therefore helping

to eliminate any droplets of liquid falling directly on the foliage. Four plants were randomly selected from each row as indicated by the circled plant number in Figure 8. Plants number 83, 87, 91, 99, 88, 92, 96, and 100 were sampled in Plots 1, 3, 5 and 8 each sampling period. The first sampling of selected plants was made on July 23, 1966, before any treatment had been made. Following the first treatment, samples were taken 6, 24 and 96 hours after each treatment for a period of three treatments. Another sample was then taken 12 hours after the last treatment in September.

Since 2,4-D has been reported to accumulate in the apical region, a sample consisted of four to six inches of apical vegetation amounting to 40 to 80 grams fresh weight. Immediately upon cutting the sample, it was placed in a plastic bag properly labeled and frozen in dry ice. Fresh weight of the sample was obtained immediately prior to placing in a Virtis freeze dryer and the dry weight was obtained immediately upon removal. The samples were then ground to pass through a twenty mesh screen in a Wiley mill and sealed in jars until chemical processing could be accomplished.

Processing of Samples

The extraction procedure used was patterned after the method used by Marquardt et al. (53). The procedure as modified for use with tomatoes and use with the electron capture detector is as follows:

1. Weigh dry ground sample to nearest 0.01 gram in a 250 milliliter Erlenmeyer flask. The dry weight of the samples varied from five to ten grams.
2. Add 25 milliliters of water.
3. Add 150 milliliters of solvent "A". (Solvent "A" = a ratio of ten

parts 10% H_2SO_4, 15 parts 95% ethyl alcohol, 25 parts petroleum ether and 75 parts ethyl ether.)

4. Place on magnetic stirrer for one hour.
5. Using a Buchner funnel, filter slurry through Whatmann No. 1 filter paper.
6. Wash residue three times with solvent "B". Bulk filtrate and washings and transfer to a separatory funnel. (Solvent "B" = ethyl ether, petroleum ether, v/v.)
7. Add 75 milliliters and three percent sodium bicarbonate solution; shake vigorously and allow to separate.
8. Discard the non-aqueous (top) layer and wash the aqueous (bottom) layer three times with 50 milliliters of petroleum ether.
9. Discard the non-aqueous layer and filter the aqueous layer through glass wool.
10. Acidify to pH of 2.9 with 10% H_2SO_4 and shake well to remove excess CO_2.
11. Extract three times with 50 milliliter portions of ethyl ether.
12. Discard the aqueous layer and bulk the non-aqueous washings.
13. Evaporate just to dryness.
14. Redissolve twice, each time in one billiliter of benzene and transfer to five milliliter flask.
15. Add four drops diazomethane.
16. Add anhydrous Na_2SO_4.

Diazomethane used for esterification of the acid of 2,4-D to the methyl ester was synthesized in the lab according to the following procedure:

1. Fit a 100 milliliter distilling flask with a dropping funnel and an

efficient condenser set downward for distillation.

2. Connect the condenser to two receiving flasks in series, with the second one containing 40 to 50 milliliters of ethyl ether. The inlet tube of the second receiver should be dipped below the surface of the ether and both receivers kept cooled in an ice bath.
3. Add five grams of KOH to the distillation flask and dissolve in eight milliliters of water.
4. Add 25 milliliters of 95% ethyl alcohol to the KOH solution.
5. Heat the flask containing the alkali solution to 65° C.
6. Add, through the dropping funnel over a period of 25 minutes, a solution of 7.0 grams of "Diazald" (N-methyl-N-nitroso-p-toluenesulfonamide) in about 130 milliliters of ethyl ether. The distillation rate should equal the rate of addition.
7. When the dropping funnel is empty, slowly add 20 milliliters of ethyl ether and continue distillation until the condensate becomes colorless.

One microliter of each sample was injected into a Varian Aerograph Hy-Fi Model 600c gas chromatograph with the Model 328 Isothermal unit attached and recorded on a Sargent Model SR recorder. The detector used was the Varian Aerograph electron capture detector with 250 millicuries of tritium adsorbed on a titanium foil. A ten-foot X 1/8 inch glass column packed with DC11 on Chromosorb W was maintained at 230° C. The detector temperature could not be monitored but assumed to be approximately 200° C. Prepurified nitrogen was used as a carrier gas at a flow rate of 21 ml/minute which was later increased to 41 ml/minute as the sensitivity of the detector decreased. The range and attention was also adjusted to maintain sensitivity of the detector. After each sample was injected once, one

milliliter of the sample was then "spiked" with ten μl of 1 X 10^{-4} grams/ml of the methyl ester of 2,4-D and then injected again to provide positive identification of the desired peak and also to provide a standard with which to determine a ratio of sample peak to standard peak for quantitative purposes as described by Marquardt et al. (53). Peak height was used for measurement of both the sample and standard peaks (43, 55). A ratio of the sample peak to the standard peak was then determined as a quantitative indication of the 2,4-D content of the sample. This ratio was then adjusted to grams of 2,4-D per gram of dry matter in the tomato vegetative tissue.

Statistical Methods

The statistical designs and models used to analyze the data in this study are as shown below (71). Missing data was calculated by the analysis of covariance (14) and Duncan's multiple range test was used for testing the various means. The following symbols are used to indicate significance: + = 90% level, * = 95% level and ** = 99% level.

I. Experiment 1 - Strawberry Harvests

A. Total yield of fruit in grams

1. Design: Split plot

2. Model:

$$Y_{ijk} = \mu + R_i + A_j + E_{ij} + B_k + (AB)_{jk} + D_{ijk}$$

Where: μ = mean
R_i = Replicates, i = 2
A_j = Treatments, j = 4
E_{ij} = Error (a) where $E_{ij} \sim$ NID $(0, \sigma E^2)$
B_k = Harvests, k = 2
$(AB)_{jk}$ = Treatment X Harvest Interaction
D_{ijk} = Error (b) where $D_{ijk} \sim$ NID $(0, \sigma D^2)$

B. Average weight per fruit in grams

1. Analysis over total yield

a. Design: Completely randomized design with unequal replication.

b. Model:

$$Y_{ij} = \mu + T_i + E_{ij}$$

Where: μ = mean
T_i = Treatments, i = 4
E_{ij} = Error, where $E_{ij} \sim NID\ (0, \sigma E^2)$
j = 9 for T_1, 9 for T_2, 7 for T_3, 4 for T_4

2. Analysis over yields following 2,4-D exposure

a. Design: Completely randomized design with equal replication

b. Model:

$$Y_{ij} = \mu + T_i + E_{ij}$$

Where: μ = mean
T_i = Treatments, i = 3
E_{ij} = Error where $E_{ij} \sim NID\ (0, \sigma E^2)$, j = 3

II. Experiment 2 - Greenbean Harvests

A. Total yield in kilograms per treatment.

1. Design: Split plot

2. Model:

$$Y_{ijk} = \mu + R_i + A_j + E_{ij} + B_k + (AB)_{jk} + D_{ijk}$$

Where: μ = mean
R_i = Replicates, i = 2
A_j = Treatments, j = 4
E_{ij} = Error (a) where $E \sim NID\ (0, \sigma E^2)$
B_k = Harvests, k = 8
$(AB)_{jk}$ = Treatment X Harvest Interaction
D_{ijk} = Error (b) where $D_{ijk} \sim NID\ (0, \sigma E^2)$

III. Experiment 3 - Tomato Harvests

A. Total yield per row in pounds

1. Design: Split plot

2. Model:

$$Y_{ijk} = \mu + A_i + B_j + E_{ij} + C_k + (BC)_{jk} + D_{ijk}$$

Where: μ = mean
A_i = Rows, i = 4
B_j = Treatments, j = 8
E_{ij} = Error (a) where E $\sim$ NID (0, σE^2)
C_k = Harvests, k = 5
$(BC)_{jk}$ = Treatment X Harvest Interaction
D_{ijk} = Error (b) where $D_{ijk} \sim$ NID (0, σD^2)

B. Number of fruit per row

1. The design and model used were the same as for III A.

2. The original data was transformed by $\sqrt{\frac{x}{2} + \frac{1}{2}}$ and the transformed data is cited in the remainder of the text.

C. Average weight per fruit in pounds

1. Design: Split plot

2. Model:

$$Y_{ijk} = \mu + B_j + E_{ij} + C_k + (BC)_{jk} + D_{ijk}$$

Where: μ = mean
A_i = Rows, i = 4
B_j = Treatments, j = 8
E_{ij} = Error (a) where $E_{ij} \sim$ NID (0, σE^2)
C_k = Harvests, k = 4
$(BC)_{jk}$ = Treatments X Harvest Interaction
D_{ijk} = Error (b) where $D_{ijk} \sim$ NID (0, σD^2)

D. Average number of seeds per gram of fruit.

1. The design and model used were the same as for III C.

IV. Experiment 4 - Analysis of the Vegetative Tissue of Tomato Plants

A. Percent dry weight of the vegetative tissue

1. Design: Split plot

2. Model:

$$Y_{ijkl} = \mu + A_i + B_j + E_{ijk} + C_l + (AC)_{il} + D_{ijkl}$$

Where: μ = mean
A_i = Treatments, i = 4
B_j = Variety, j = 2
E_{ijk} = Error (a) where $E_{ijk} \sim$ NID $(0, \sigma E^2)$, k = 4
C_l = Sampling time, l = 11
$(AC)_{il}$ = Treatment X Sampling time Interaction
D_{ijkl} = Error (b) where $D_{ijkl} \sim$ NID $(0, \sigma D^2)$

B. Ratio of 2,4-D content in the vegetative tissue

1. The design and model used were the same as for IV A.

RESULTS AND DISCUSSION

Visual Symptoms of Injury

1966 observations

Variation in time of appearance of 2,4-D injury in the ambient atmosphere was observed between older established plants in the Horticulture Vineyard and the newly planted plants in the outdoor plots (Treatment 4). By mid-June the older plants showed visible symptoms and as early as July 1 they showed severe injury (Plate Ia). However, by July 7, none of the newly planted grapevines showed any visual symptoms of 2,4-D injury (Plate Ib).

By mid-July most heat damage due to the electrical power failure, had become inconspicuous and growth of the plant material in the houses, especially the tomatoes, was quite abundant (Plate Ic); however, the growth rate was not as great on the outdoor plots (Plate Id).

By July 13, slight 2,4-D injury began to appear on the grapes and tomatoes in Treatments 3 and 4 and from July 15 to 22, definite injury became apparent (Plate Ie). In general, injury of the tomatoes appeared to be greater in Treatment 3 than in Treatment 4 although both received their exposure from ambient atmosphere. This is perhaps due to the fact that the plants were in a more succulent state of growth in the houses and would therefore be more susceptible to injury than the slightly more hardened off plants in Treatment 4. Guzman (36) has shown a relationship of 2,4-D injury to temperature which applies in this case as the houses were warmer and would therefore tend to enhance the injurious effects of the 2,4-D. Although Treatment 2 was a presumably 2,4-D-free atmosphere, very

slight leaf modification appeared occasionally on the tomatoes (Plate If), however, it was not at all comparable to the degree of injury observed in Treatment 3 (Plate Ig). No injury was observed before mid-July on the grapes in Treatments 1 and 2 (Plate Ih) and no injury was observed on the strawberries or raspberries in any of the treatments up to this time.

No discernible injury was apparent on any of the tomatoes in Treatment 1 immediately following or at 96 hours after the first exposure for six hours to one ppb of the butyl ester of 2,4-D (Plate Ii). Treatments 3 and 4 continued to show considerable injury (Plates Ij, Ik). Within 24 hours after the second exposure to the butyl ester, 2,4-D injury symptoms became apparent in Treatment 1. Bending and cupping of leaves on the apical portions of the plant was the major effect with slight epinasty of stems on some plants (Plate Il).

By August 4, 72 hours after the second exposure, injury was readily distinguishable on the tomatoes in Treatment 1. The injury consisted of epinasty of terminal shoots with rolling and cupping of leaves. By August 6, injury on the tomatoes had increased to the point where it was similar in form but somewhat more severe than that found in Treatment 3. Injury to the grape plants was not yet visible in Treatments 1 and 2 (Plates IIa, IIb). In Treatment 3 only a slight discoloration of the most terminal grape leaves appeared, but in Treatment 4, tubiform leaves were formed and considerable vein clearing was apparent (Plates IIc, IId). This is in agreement with early symptoms from sublethal concentrations of 2,4-D found by Daines (20) on grapes and tomatoes, and by Gorter and Van Der Zweep on many other species. The strawberries and raspberries showed no apparent injury in any of the treatments.

Plate I. Visual symptoms of injury

a. Grape - Horticultural Vineyard - July 1, 1966

b. Grape - Treatment 4 - July 1, 1966

c. Tomato - Treatment 1 - July 22, 1966

d. Tomato - Treatment 4 - July 22, 1966

e. Grape - Treatment 4 - July 22, 1966

f. Tomato - Treatment 2 - July 22, 1966

g. Tomato - Treatment 3 - July 22, 1966

h. Grape - Treatment 1 - July 22, 1966

i. Tomato - Treatment 1 - July 29, 1966

j. Tomato - Treatment 3 - July 29, 1966

k. Tomato - Treatment 4 - July 29, 1966

l. Tomato - Treatment 1 - August 2, 1966


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Twenty-four hours after the third exposure on August 9, severe epinasty was apparent on the tomatoes throughout Treatment 1. All terminal and immature leaves were curled, first longitudinally and then down the midrib (Plate IIe). Only the very young apical shoots showed slight epinasty in Treatment 2 while Treatments 3 and 4 continued to show leaf rolling and wrinkling, including some of the more mature leaves. On August 12, 96 hours after the third exposure, definite cupping and some vein clearing was first apparent on the grapes in Treatment 1. This two weeks delay in appearance of symptoms and the less severity of injury compared to the tomatoes can perhaps be attributed to the immaturity and greater succulence of the tomatoes than the grapes at the time of the first exposure. A comparison of the injury sustained by the two species as of August 26 is shown in Plate IIe through Plate III. According to Daines (20), plant susceptibility to sublethal exposures of 2,4-D is markedly influenced by the growth condition of the plant and also by environmental factors. The grape plants began developing much earlier in the season than the tomato plants so that at the time of the first 2,4-D exposure in Treatment 1 the grapes and tomatoes differed greatly in their physiological maturity. Injury to the grapes in Treatments 3 and 4 had been prominent previously which would coincide with an ambient exposure earlier in their development.

After five exposures to 2,4-D (August 26), severe epinasty, leaf curling and vein clearing remained prominent on the tomatoes in Treatment 1. In Treatment 3, injury had increased so that it was again similar in form and almost in severity to that of Treatment 1 (Plates IIe - IIh). Grape injury in Treatment 1 was at this time more severe than in Treatment 3 but less severe than in Treatment 4 (Plates IIi - III).

After seven exposures (September 9), some of the tomato flower blossoms became dry and began dropping off, while others remained attached even after nine exposures (Plate IIIa). This can possibly be explained on the basis of an exposure during a critical period of flower development. Derscheid (22) found the application of 2,4-D at the time of anthesis to be very detrimental. If the flowers are more tolerant at certain stages of growth and if a six hour exposure of 2,4-D occurred during a very intolerant stage, this could account for the severely injured flowers while others showed little or no injury. Tukey et al. (75) also found that flower development was arrested by application of 2,4-D while the plants were growing vigorously. At this time, injury, manifested by wrinkled, cupped and slightly discolored leaves, also became readily apparent in Treatment 2 (Plate IIIb). This would thus indicate that although the activated charcoal filters adsorbed most of the ambient 2,4-D taken in by the fans, a small portion must have passed through to permit a slow accumulation to an injurious concentration in the vacuoles of active parenchyma calls, as has been shown by several investigators (4, 16, 17, 29). This was also apparent in the uneven ripening of grape berries which was evident in both Treatments 1 and 2 (Plates IIIc, IIId). Since the fruit acts as an active sink for transport of food and 2,4-D (17), it serves as a good indicator of the presence of very low concentrations of 2,4-D in the atmosphere as it will accumulate the compound to a concentration in which visible symptoms will be produced. Visible injury to the grape foliage at the end of the season was severe in Treatment 1, very severe in Treatments 3 and 4 and very slight in Treatment 2 (Plates IIe - IIIh). Although the tomatoes and grapes exhibited severe symptoms in Treatments 1, 3, and 4,

Plate II. Visual symptoms of injury

a. Grape - Treatment 1 - August 6, 1966

b. Grape - Treatment 2 - August 6, 1966

c. Grape - Treatment 3 - August 6, 1966

d. Grape - Treatment 4 - August 6, 1966

e. Tomato - Treatment 1 - August 12, 1966

f. Tomato - Treatment 2 - August 12, 1966

g. Tomato - Treatment 3 - August 12, 1966

h. Tomato - Treatment 4 - August 12, 1966

i. Grape - Treatment 1 - August 26, 1966

j. Grape - Treatment 2 - August 26, 1966

k. Grape - Treatment 3 - August 26, 1966

l. Grape - Treatment 4 - August 26, 1966


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Plate III. Visual symptoms of injury

a. Tomato - Treatment 1 - September 9, 1966

b. Tomato - Treatment 2 - September 9, 1966

c. Grape - Treatment 1 - September 22, 1966

d. Grape - Treatment 2 - September 22, 1966

e. Grape - Treatment 1 - September 22, 1966

f. Grape - Treatment 2 - September 22, 1966

g. Grape - Treatment 3 - September 22, 1966

h. Grape - Treatment 4 - September 22, 1966

i. Strawberry - Treatment 1 - September 22, 1966

j. Raspberry - Treatment 1 - September 22, 1966

k. Raspberry - Treatment 2 - September 22, 1966

l. Raspberry - Treatment 4 - September 22, 1966


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no injury was noted on the strawberries or raspberries throughout the growing season (Plates IIIi - IIIl).

1967 observations

In 1966, 2,4-D symptoms did not appear on the newly planted grapes in Treatment 4 until after mid-July, however, in 1967, slight injury was apparent by June 29. This is not attributed to an earlier build up of 2,4-D in the ambient atmosphere but rather an effect of the residual 2,4-D left in the plant tissue from the previous year. This is in agreement with the previous year's observations as well as those of other investigators (10, 13). In 1967, 2,4-D injury symptoms were apparent on the tomatoes in Treatment 1, 96 hours after the first exposure (Plates IVa - IVd) which is in contrast to 24 hours after the second exposure in 1966. The 2,4-D was released three weeks earlier in 1967, therefore exposing the plants in a more immature state of development. A very slight amount of leaf twisting was observed in Treatment 2 and a greater amount of tomato injury was again observed in Treatment 3 than Treatment 4.

By July 7, no distinguishable grape injury had occurred in Treatments 1 and 2 and only a slight discoloration and very slight cupping of the leaves was apparent in Treatment 3 (Plates IVe - IVg). Treatment 4, however, showed a considerable amount of leaf roll, cupping and vein clearing (Plate IVh). At this time, no injury was apparent on the greenbeans in any of the treatments.

By July 21, after the third exposure, leaves on the upper 8-12 inches of the tomato plants in Treatment 1 sustained severe epinasty, leaf roll and cupping. Those in Treatment 2 continued to show very slight twisting

Plate IV. Visual symptoms of injury

a. Tomato - Treatment 1 - July 7, 1967

b. Tomato - Treatment 2 - July 7, 1967

c. Tomato - Treatment 3 - July 7, 1967

d. Tomato - Treatment 4 - July 7, 1967

e. Grape - Treatment 1 - July 7, 1967

f. Grape - Treatment 2 - July 7, 1967

g. Grape - Treatment 3 - July 7, 1967

h. Grape - Treatment 4 - July 7, 1967

i. Tomato - Treatment 1 - July 21, 1967

j. Tomato - Treatment 2 - July 21, 1967

k. Tomato - Treatment 3 - July 21, 1967

l. Tomato - Treatment 4 - July 21, 1967


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and bending of the apical leaves. Tomato plants in Treatment 3 showed considerable twisting and cupping of the upper leaves, and Treatment 4 showed a slight amount of epinasty but again less than Treatment 3, (Plate IVi - IVl). The tomato plants in Treatment 4 were hardened-off somewhat by the cold wet weather during the month of June which resulted in a very cold soil in which little or no growth took place for about two to three weeks. By July 21, grape injury was prominent in three of the treatments and was manifested mainly through discoloration and cupping in Treatment 1 and discoloration, cupping and vein clearing in Treatment 3 (Plates Va - Ve). Injury in Treatment 4 was very severe at this time resulting in extreme vein clearing and the formation of long, narrow tubiform leaves (Plate Vd).

By August 12, at which time five exposures had been made, injury had increased sharply on the tomatoes and grapes in Treatment 1. Longitudinal folding of the leaf with subsequent rolling of the midrib was prominent on the upper one-fourth of all tomato plants. Treatment 2 continued to show some slight twisting and curling of the youngest leaves and in Treatment 3 the amount of injury was increased somewhat and remained more severe than that found in Treatments 2 or 4 (Plates Ve - Vh). Severe injury to the grapes in Treatment 1 had become prominent by August 26 through cupping, vein clearing and formation of tubiform leaves. No injury of grapes was apparent in Treatment 2 and the injury in Treatment 3 was very similar to Treatment 1 (Plates Vi - Vk). Injury in Treatment 4 increased in severity with the deformation of leaves and extreme vein clearing (Plate Vl).

Although bean seedlings readily exhibit 2,4-D injury symptoms at concentrations as low as 25 ppm (11, 24), no injury was observed on the

Plate V. Visual symptoms of injury

a. Grape - Treatment 1 - July 21, 1967

b. Grape - Treatment 2 - July 21, 1967

c. Grape - Treatment 2 - July 21, 1967

d. Grape - Treatment 4 - July 21, 1967

e. Tomato - Treatment 1 - August 4, 1967

f. Tomato - Treatment 2 - August 4, 1967

g. Tomato - Treatment 3 - August 4, 1967

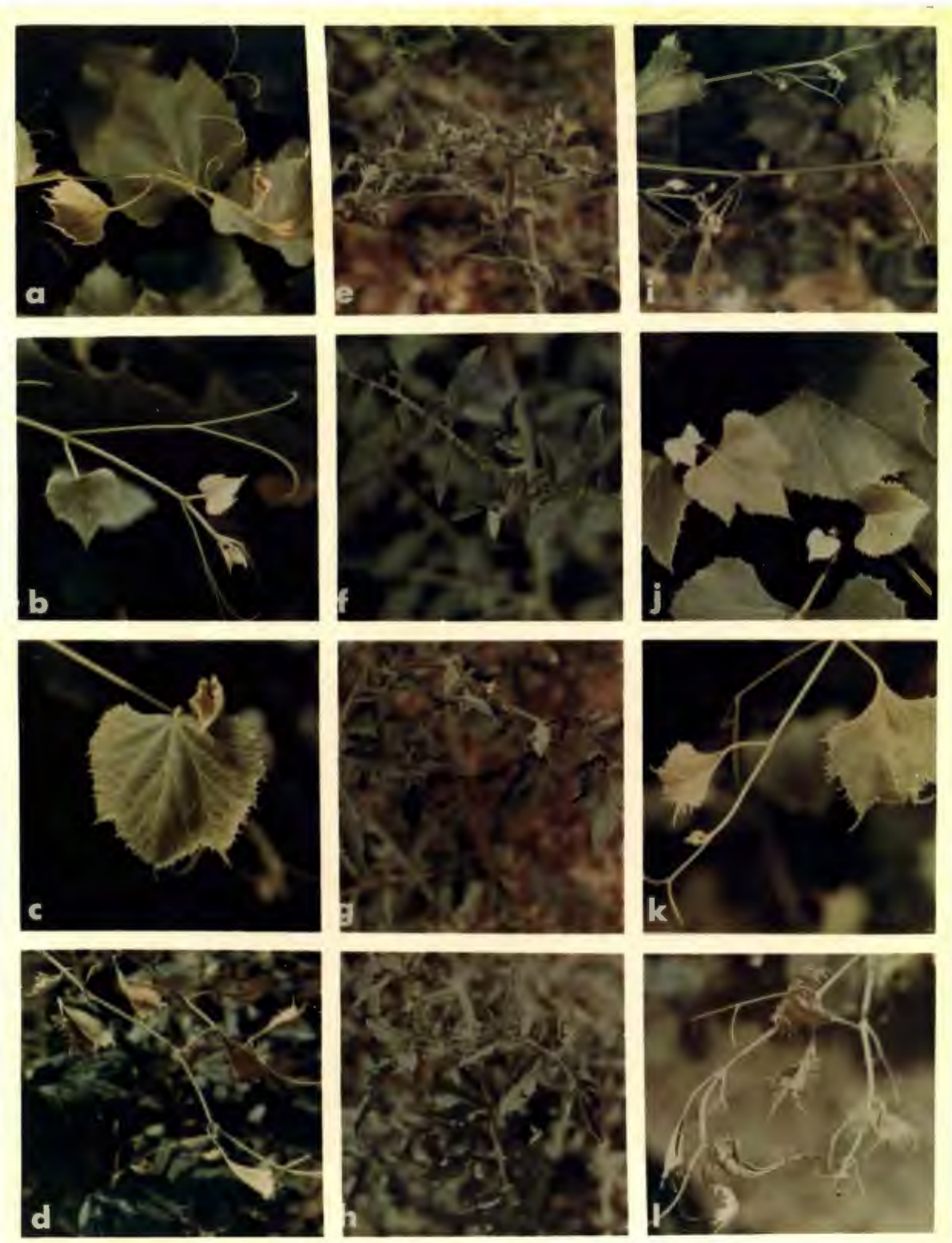
h. Tomato - Treatment 4 - August 4, 1967

i. Grape - Treatment 1 - August 4, 1967

j. Grape - Treatment 2 - August 4, 1967

k. Grape - Treatment 3 - August 4, 1967

l. Grape - Treatment 4 - August 4, 1967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greenbeans in any of the treatments throughout the growing season. Neither was injury observed on the raspberries or strawberries for the duration of the season.

As a result of these observations, it is obvious that the stage of plant development at the time of 2,4-D exposure is a vital factor in determining the time of response and the degree of injury. The more immature and succulent a plant is, the quicker it will respond and with greater severity. This response is in agreement with the work of other investigators (20, 22, 39, 67) and adds impetus to Derschied's statement that "the stage of growth is the most important factor in response of a plant to 2,4-D" (23). It is also apparent that the history of the plant can be important in determining a plant's response. In 1966, newly planted grape plants were not injured as soon or as severely as plants in the vineyard with several years of injury. The grape plants in Treatment 1 in 1966 were not injured as severely as Treatment 4 and as a result, in 1967 did not show injury as soon as Treatment 4. This is therefore indicative of a residual parent molecule of 2,4-D or an active degradation product remaining in the tissue throughout its dormant period.

Depending on the stage of growth, tomatoes and grapes appeared to be similar in their degree of sensitivity to 2,4-D. Although injury appeared first and was most severe on the grapes in Treatment 4 and injury appeared first and was most severe on the tomatoes in Treatment 1, this difference can probably be explained on the basis of different growing conditions resulting in more or less succulent growth and also on the basis of different times of exposure. Few additional symptoms and little increase in severity of injury occurred during tomato fruit development. As cited

in the literature, (16, 17) this would be due to the high transport of carbohydrates and 2,4-D to the active sink established by the fruit and therefore a diversion from the leaves. This is also in agreement with Williams et al. (82) who reported that tomatoes, while ruined commercially at low concentrations of 2,4-D, were not easily killed upon reaching a height of 10-12 inches.

The strawberry and raspberry plants were tolerant of the amount of 2,4-D in the ambient atmosphere and of the 1 ppb 2,4-D ester released to the atmosphere in Treatment 1 and therefore exhibited no visible symptoms of injury throughout the two growing seasons.

It is apparent from these observations that 2,4-D spray drift and volatility can cause severe injury at concentrations of 1 ppb and less. Based on the comparisons of injury it is also concluded that the concentration of volatile 2,4-D in the atmosphere in the vicinity of the study was much less than 1 ppb as, although severe injury occurred outdoors on the grapes and also in Treatment 1, a great difference in duration of exposure existed between the two. Treatment 4 received "continuous exposure" while Treatment 1 was exposed for a period of only six hours out of 168. It is, however, likely that the ambient 2,4-D was higher in concentration at certain periods than others and could have therefore produced the injury during that period of time.

Strawberry Harvests

Total yield

Total strawberry yields in 1966 and raspberry yields in 1966 and 1967 were not sufficient to provide information concerning the treatment

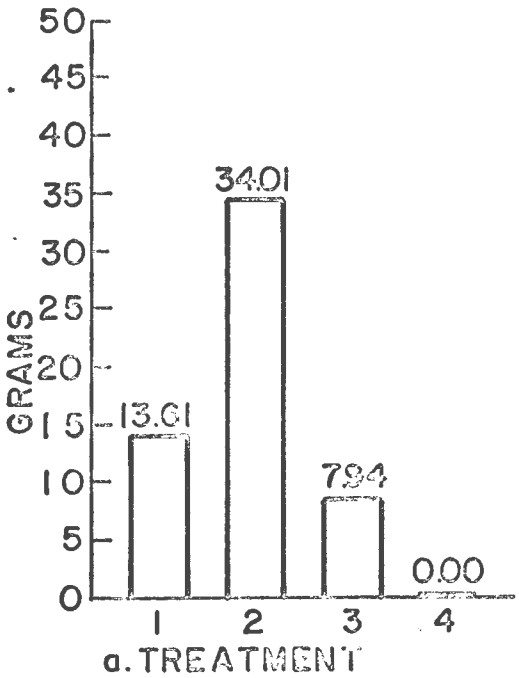
effects. In 1967, five strawberry harvests were made, three of which were completed prior to the time of releasing 2,4-D in Treatment 1. Therefore, the two harvests receiving the exposures to 2,4-D in Treatment 1 (designated at Harvests 4 and 5) were analyzed separately for any existing treatment effects. The analysis of variance (Table 1) shows harvests significant at the 90% level and the treatment X harvest interaction significant at the 95% level. From the table of means (Table 2) it is apparent that the interaction is due to the fact that the treatments are not behaving similarly over both harvests. It is indicative that the 2,4-D exposure could have caused a concentration of fruit set in the fourth harvest and a reduction in yield for Harvest 5 because of the concentrated previous harvest and also possibly because of an inhibition of fruit set or flower initiation. The reduction in yield in Treatment 1 of Harvest 5 may also be due to the effect of the greater number of exposures to 2,4-D and also the stage of growth of the plants prior to the fifth Harvest. Derscheid (22) has reported that if growth is rapid, differentiation is slow and yield reduction is slight, however, yield can be reduced by several exposures. If the growth rate is slow, differentiation is more rapid and 2,4-D applied at this time caused larger yield reductions. Both of those factors coincide with existing conditions at the time of Harvest 5 and therefore may contribute to the trend of reduction in yield in Treatment 1 by a calibrated exposure to 2,4-D and by ambient 2,4-D in Treatment 3. This trend toward a reduction in yield, although quite apparent from the treatment means (Figure 12a) was not statistically significant. This is due to the existence of a large error term in the experiment and also possibly to a masking effect of the treatment produced by the interaction with harvests.

Table 1. Analysis of variance for strawberry yields in grams for 1967

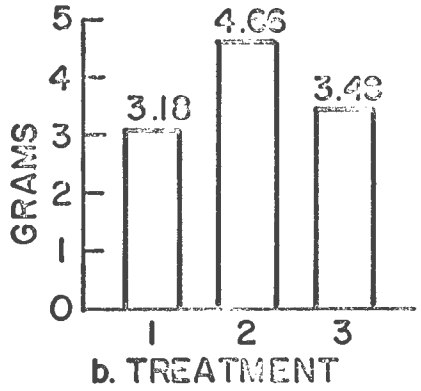
Source of Variation	Degrees of Freedom	Sums of Squares	Mean Squares	F
Replicates	1	209.35	289.35	
Treatments	3	2534.24	844.74	3.72
Error (a)	3	682.85	227.16	
Harvests	1	680.18	680.18	7.67 +
Treatments X Harvests	3	3028.01	1009.33	11.38 *
Error (b)	4	334.59	88.65	
Total	15	7549.22		

Table 2. Treatment and harvest means of strawberry yields in grams for 1967

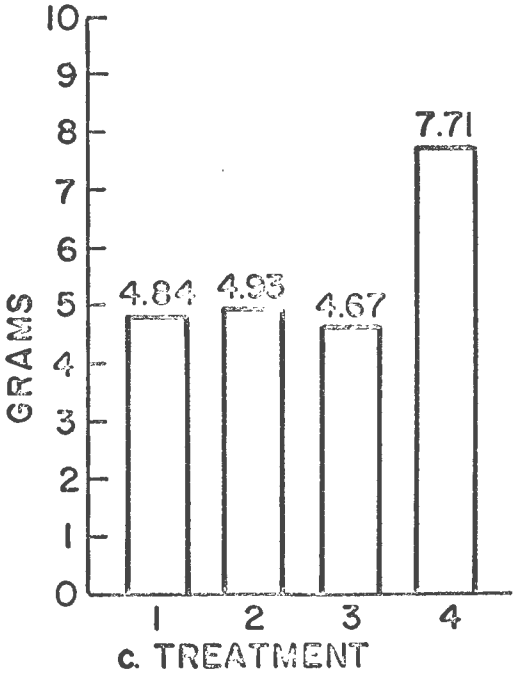
	Harvest 4	Harvest 5	Treatment Means
Treatment 1	20.42	6.80	13.61
Treatment 2	4.54	63.50	34.01
Treatment 3	4.54	11.34	7.94
Treatment 4	0.00	0.00	0.00
Harvest Means	7.37	20.41	



a. Strawberry yields for harvests exposed to 2,4-D



b. Fruit size of strawberries for harvests exposed to 2,4-D



c. Fruit size of strawberries for all harvests

Figure 12. Treatment means for strawberry harvests

The absence of yield in Treatment 4 is believed due to somewhat adverse growing conditions, mainly a cooler air temperature as well as a cooler, wetter and poorly aerated soil. Additional replication of both treatment and harvests would decrease both error terms involved and would therefore provide a more powerful test for describing the treatment effects.

Size of fruit

Although a slight difference in size of fruit occurred between treatments this difference was not significant at the 90% level (Table 3). The treatment means (Table 4) of fruit size as shown in Figure 12b indicates a trend for larger fruit to be produced in the atmosphere containing the least 2,4-D. This observation should be correlated with the apparent deformation of the fruit which was quite obvious in the later harvests of all three treatments but somewhat more prominent in Treatments 1 and 3. Holt (39) observed suppression of organogenesis of the floret of oats treated with butyl ester of 2,4-D and that malformation and abortion of floret organs occurred during organogenesis. He also noted that the ovule fails to develop in florets which are treated during the initiation of the ovule and that it may proliferate and abort. This could possibly be happening in the strawberry causing abortion of some of the ovules in each aggregate. It should be considered that although volatile 2,4-D in the atmosphere is apparently a factor, a pronounced environmental influence is created through the use of the plastic greenhouses. In the earlier harvests, when some production did occur in Treatment 4, fruit size was significantly different among treatments (Table 5). Duncan's multiple range test shows that the fruit was larger in Treatment 4 than any of the other treatments

Table 3. Analysis of variance for fruit size of strawberries in grams for harvests exposed to 2,4-D in 1967

Source of Variation	Degrees of Freedom	Sum of Squares	Means Squares	F
Treatments	2	3.69	1.84	1.30
Error	6	8.47	1.41	
Total	8	12.16		

Table 4. Treatment means of fruit size of strawberries in grams for harvests exposed to 2,4-D in 1967

	Rep. 1	Rep.2	Rep. 3	Treatment Means
Treatment 1	2.72	2.27	4.54	3.18
Treatment 2	3.18	4.90	5.90	4.66
Treatment 3	3.18	2.72	4.54	3.48

and that fruit size was quite similar within the houses (Figure 12c). The smaller fruit size within the houses is perhaps due to less than optimum pollination conditions resulting in fewer pistils within the aggregate being pollinated and also possibly to the increased vegetative growth and reduced fruit growth in the warmer atmosphere of the enclosed structures.

It is concluded that although the yield and fruit size of strawberries was not significantly reduced by the volatility of 2,4-D at the concentrations used in this study, a trend did exist in this direction.

Table 5. Analysis of variance for fruit size of strawberries in grams for all harvests in 1967

Source of Variation	Degrees of Freedom	Sums of Squares	Means Squares	F
Treatments	3	79.76	25.58	5.96 **
Error	25	111.46	4.46	
Total	28	191.22		

Duncan's Multiple Range Test of Treatments Treatment Means:

4	2	1	3
7.71	4.93	4.84	4.67

Many of the yield reductions reported in the literature (13, 22, 39) were obtained at somewhat higher concentrations of 2,4-D and in most cases it resulted from the use of a spray rather than spray drift or volatility. Perhaps if the exposures to 2,4-D had been made at an earlier stage of growth or during the time of anthesis, a more pronounced effect would have been evident.

Greenbean Harvests

Yields of greenbeans were significantly different at the 90% level among treatments and at the 99% level among harvests (Table 6). No interaction was apparent between the two variables. With the exception of the first harvest on Treatments 1, 2 and 3, the yields of all treatments

Table 6. Analysis of variance for greenbean yields in kilograms for 1967

Source of Variation	Degrees of Freedom	Sums of Squares	Mean Squares	F
Replicates	1	0.03	0.03	
Treatments	3	3.36	1.12	6.22 +
Error (a)	3	0.53	0.18	
Harvests	7	20.24	2.89	13.76 **
Treatments X Harvests	21	5.61	0.27	1.28
Error (b)	28	5.82	0.21	
Total	63	35.59		

Duncan's Multiple Range Test of Treatments Treatment Means:

1	2	3	4
1.14	1.13	1.04	0.58

tended to follow a normal yield curve, resulting in an abrupt increase in yield which climaxed at Harvest 4. Treatments 1, 2 and 3 all produced high yields on the first Harvest, while Treatment 4 produced very little, however, after Harvest 2, all treatments followed the same trend.

It is suggested that the differences obtained among harvests may be explained on the basis of a combination of three factors. Perhaps the most important factor is the normal physiological production and aging of the plants. Secondly, is the variation caused by the environmental conditions

between the plastic houses and the outdoor plots which resulted in a delayed and decreased yield in Treatment 4 due to cool, wet soil and air conditions. A third factor would be the time intervals between harvests as they varied from harvest to harvest depending on the quality and quantity of available beans for food processing.

Although Duncan's multiple range test does not show Treatments 1, 2, and 3 significantly different, an interesting relationship exists between Treatment 1 and Treatments 2 and 3. Treatment 1 had the largest yield at the first Harvest at which time it had received two exposures to 2,4-D. At the second Harvest, which includes one more exposure, it was still the highest, but on the third and fourth Harvests, it was the lowest of the three. This as with the strawberries could be indicative of the fruit set enhancement by one or two exposures, but detrimental effects with additional exposures.

Since only Treatment 4 was declared significantly different from the other treatments (Figure 13) it is felt that the variation in treatments is due mainly to environmental conditions and it is therefore concluded that these conditions were probably more responsible for the reduction in yield of greenbeans than the effects of volatile 2,4-D in the atmosphere.

Tomato Harvests

Total yield per row

In this experiment, rows, treatments, harvests and the treatment X harvest interaction were all significant at the 99% level (Table 7). Rows consisted of two variables; variety and distance from the source of 2,4-D, which were therefore confounded with the exception that Row 1 and Row 3

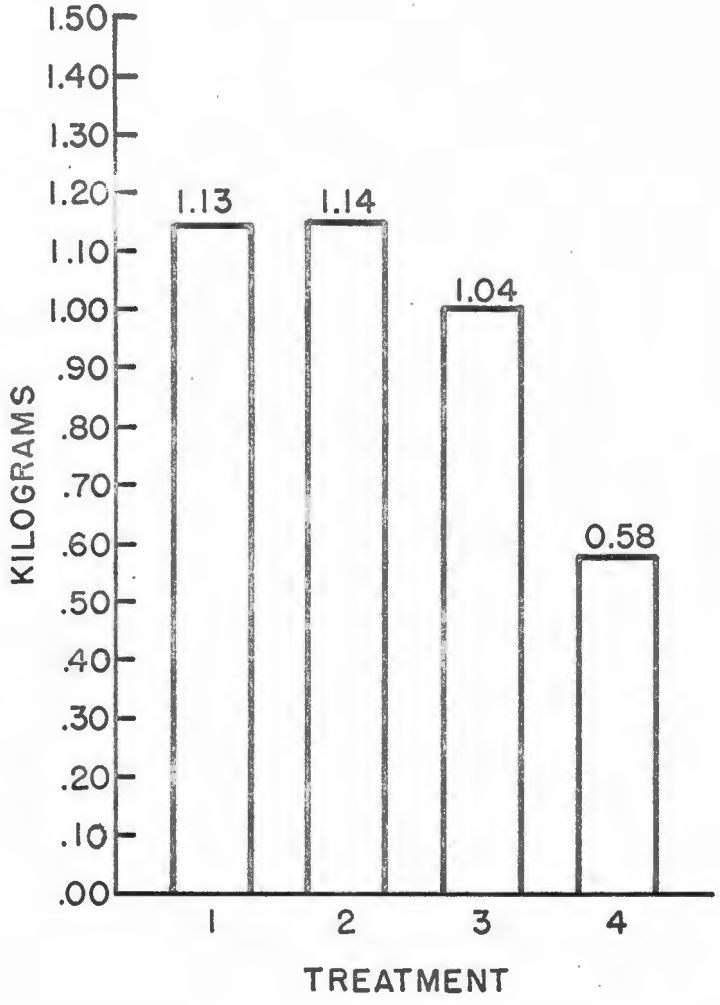


Figure 13. Treatment means for greenbean harvests

were both the Campbell 1327 variety. Because of this it is felt that some information can be obtained regarding varietal differences with the distance from source. As shown in Table 7 and Figure 14a, Row 2, variety Avalanche, and Row 3, variety Campbell 1327, are not significantly different from each other but are significantly different from the other two rows. Yields from Row 4, variety Manapal, were significantly higher than all other rows even though it was the closest row to the source of 2,4-D release. It is felt that distance from source may be a factor mainly concerning the effects due to droplets of spray or spray drift rather than volatility. Although not readily distinguishable through visual symptoms, these data confirm Weigle's[1] observations that the Manapal variety (Row 4) was more tolerant to volatile 2,4-D than Avalanche (Row 2) which had a considerably lower yield even though Row 2 was farther from the source of contamination. Although both Rows 1 and 3 were Campbell 1327, the yields from Row 1 were significantly higher than the yields from Row 3. This can probably be attributed to the effects of distance from source with the greater distance producing the higher yields.

As shown in Table 7 and Figure 14b, a considerable variation exists among treatments. The total yield relationship appears to be the inverse of that found in the strawberries and greenbeans, in that yields in Treatments 1 and 3 are slightly higher than Treatment 2. However, the individual treatment yields per harvest of tomatoes tends to coincide with the strawberry and greenbean harvests in that the greater yield in Treatment 1

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[1]Weigle, J. L. Iowa State University, Ames, Iowa. Data from field study on tomatoes. Private communication. 1966.

Table 7. Analysis of variance for total yield of tomatoes in pounds

Source of Variation	Degrees of Freedom	Sums of Squares	Mean Squares	F
Rows	3	18,335.43	6,111.81	56.93 **
Treatments	7	5,039.10	719.87	6.70 **
Error (a)	21	2,254.37	107.35	
Harvests	4	59,743.24	14,935.81	22.81 **
Treatments X Harvests	28	43,023.19	1,536.54	2.35 **
Error (b)	96	62,865.88	654.85	
Total	159	191,261.25		

Duncan's Multiple Range Tests

Rows: (99%)

4	1	2	3
45.83	28.93	19.99	19.25

Treatments: (99%)

3	1	2	4
35.27	30.81	28.71	20.25

Harvests: (99%)

5	4	3	2	1
57.30	44.58	21.83	11.02	7.78

can be attributed to greater production during the first three harvests, which is possibly again due to an enhancement of flower initiation or fruit set through the auxin-like properties of 2,4-D in the first few exposures and to a detrimental effect from additional exposures. It was noted also in 1967, that more fruit was ripe earlier in Treatment 1 than in any of the other treatments. The significantly higher yield in Treatment 3 could probably be due to a more beneficial or less detrimental effect on flower initiation and fruit set produced by a very low concentration of ambient 2,4-D as a continuous exposure rather than a short time and somewhat higher concentrated exposure as in Treatment 1. Wittwer and Bukovac (83) have reported a stimulation of early flowering and a reduction in yield of tomatoes on the later developing fruit clusters. This effect would also contribute to the higher initial yield but lower yield from the later harvests as was observed in Treatment 1 (Figure 15).

It has been demonstrated (48) that the capacity for tomato fruit set is dependent upon temperature and that this effect is usually detrimental at temperatures above 75° F. Average temperatures within the greenhouses were above this optimum range, however, it is felt that this, along with increased humidity, provided improved overall growing conditions which subsequently enhanced the yield of tomatoes within the houses. The decrease in yield in Treatment 4 is attributed to less than optimum environmental conditions compared to that within the houses. It was, therefore, not as favorable for growth and production.

Harvests are significant (Table 7, Figure 14c) as a result of a normal physiological yield response with Harvest 5 being the largest because all remaining fruit on the plants were harvested at this time with the

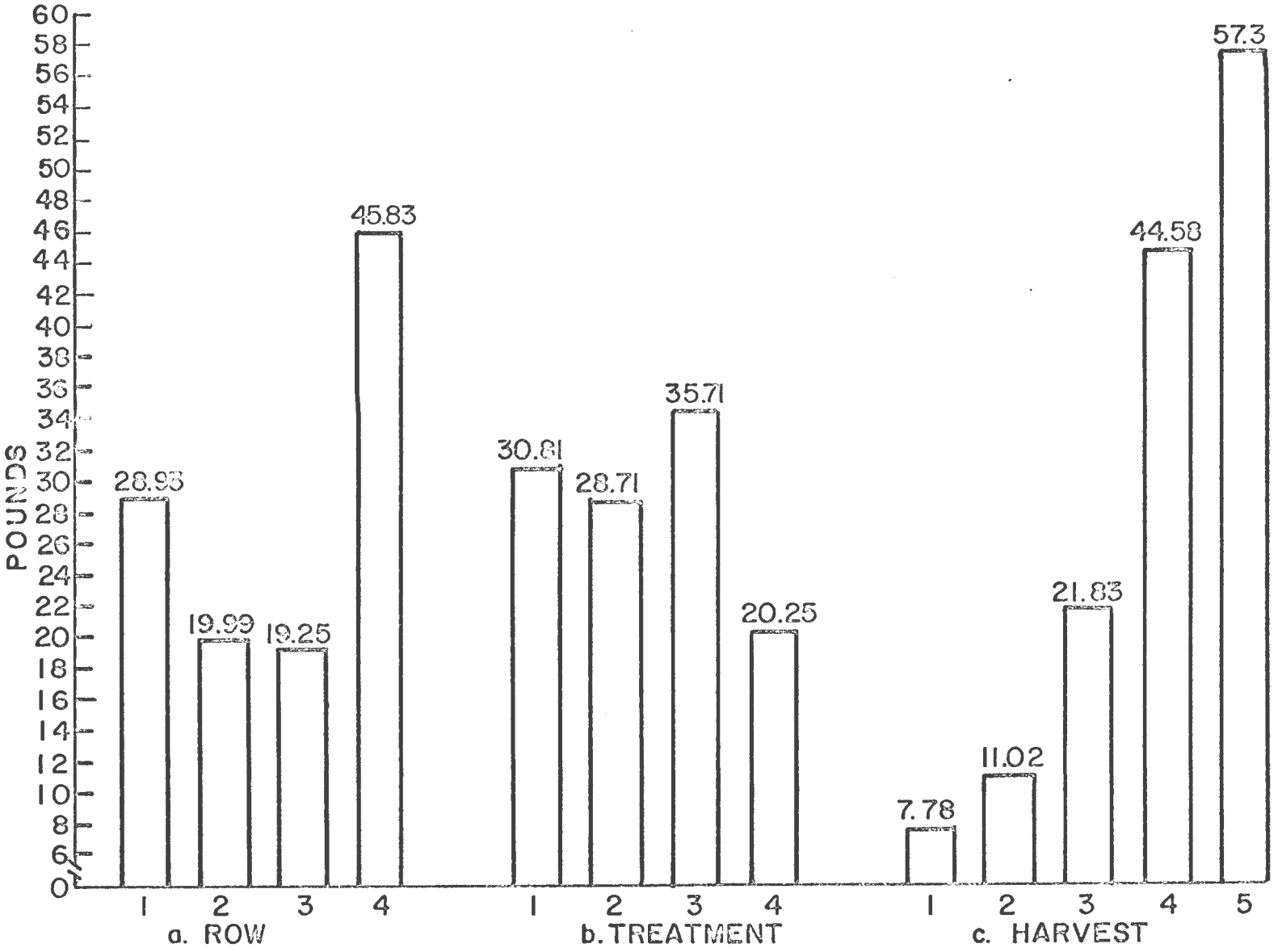

Figure 14. Means for total yield of tomatoes

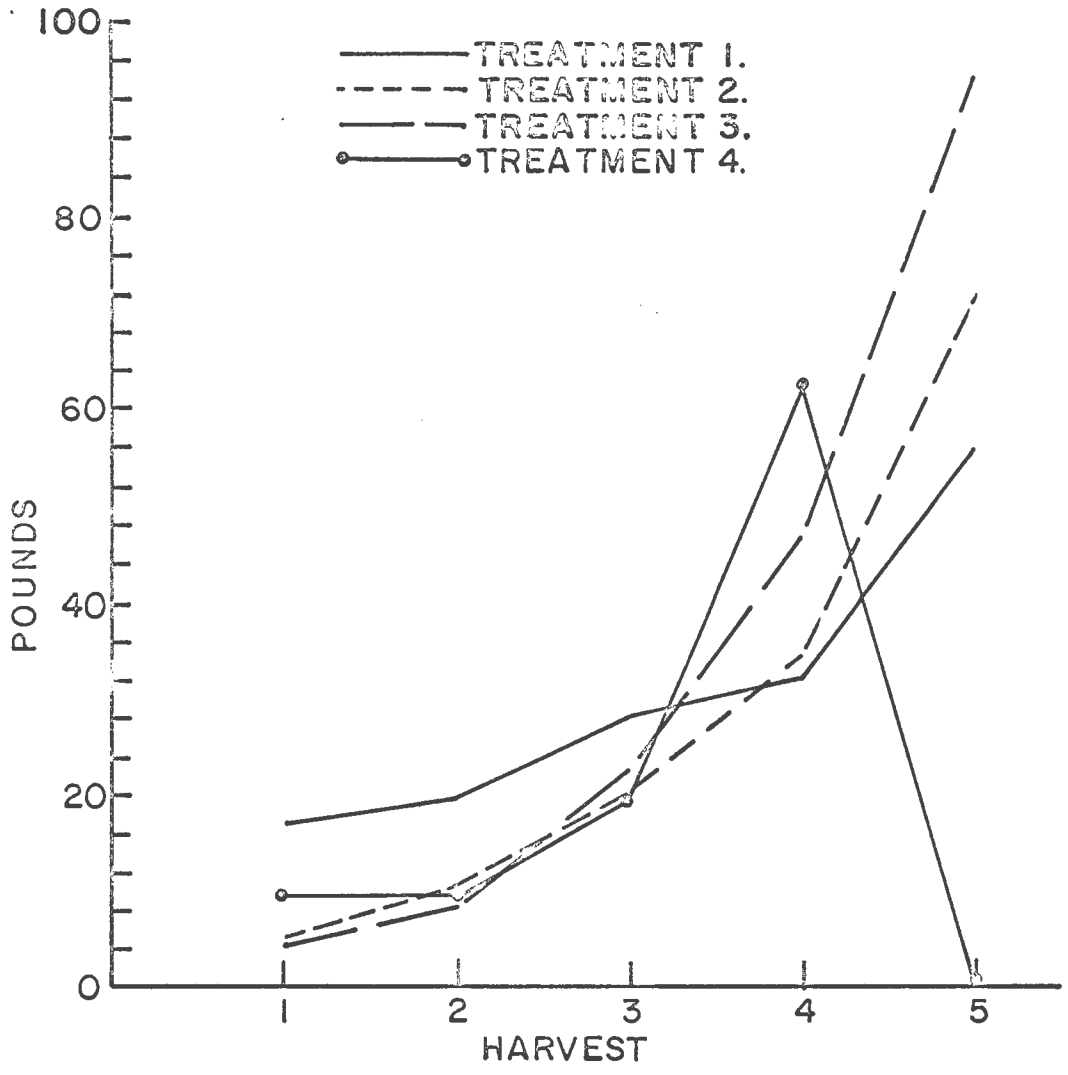

Figure 15. Treatment X harvest interaction for total yield of tomatoes

exception of Treatment 4 in which all remaining fruit was harvested in the fourth crop. This difference in harvest completion dates, as well as the higher earlier yield in Treatment 1 is responsible for the significance of the treatment X harvest interaction (Figure 15).

It is concluded that the significant differences between treatments is mainly a result of environmental effects since most of the variation lies between Treatment 4 and Treatments 1, 2 and 3, and also since no significant difference occurred between the plants in the atmosphere with the least 2,4-D and the atmosphere in which 2,4-D was released. This also coincides with yield observations on other species in this study.

Number of fruit per row and average weight per fruit

The number of fruit produced per row by the Manapal variety was significantly higher than any of the other varieties (Table 8, Figure 16a) yet the average weight per fruit of this variety was not significantly smaller than any of the other varieties (Table 9, Figure 17a). Variety Avalanche produced the smallest fruit and Campbell 1327, in the row farthest from the source of 2,4-D release, produced the largest fruit. There was not, however, any significant difference in size of fruit between rows.

Treatments were significantly different at the 99% level for both number of fruit per row and fruit size (Tables 8 and 9). The two measurements varied inversely with each other over treatments in which the lowest number of fruit and the largest fruit were produced in Treatment 4 (Figure 16b and 17b). Although the number of fruit produced was not significantly different between Treatments 2 and 3, significantly larger fruit was

Table 8. Analysis of variance for the number of tomato fruit per row

Source of Variation	Degrees of Freedom	Sums of Squares	Mean Squares	F
Rows	3	185.69	61.90	18.10 **
Treatments	7	709.06	101.29	29.62 **
Error (a)	21	71.86	3.42	
Harvests	4	3206.22	801.56	53.75 **
Treatments X Harvests	28	2570.02	91.79	6.16 **
Error (b)	96	1431.50	14.91	
Total	159	8174.36		

Duncan's Multiple Range Tests
Rows: (99%)

Row 4	Row 1	Row 2	Row 3
11.85	10.40	9.74	8.90

Treatments: (99%)

T3	T2	T1	T4
12.22	11.08	10.53	6.80

Harvests: (99%)

H5	H4	H3	H2	H1
16.64	13.85	9.53	6.45	4.65

Table 9. Analysis of variance for the average weight of tomato fruit in pounds

Source of Variation	Degrees of Freedom	Sums of Squares	Mean Squares	F
Rows	3	0.031	0.010	1.11
Treatments	7	0.312	0.044	4.89 **
Error (a)	21	0.196	0.009	
Harvests	3	0.392	0.131	22.032 **
Treatments X Harvests	21	0.265	0.013	2.124 *
Error (b)	72	0.428	0.006	
Total	127	1.624		

Duncan's Multiple Range Tests

Treatments: (99%)

T4	T1	T2	T3
0.33	0.28	0.24	0.22

Harvests: (99%)

H1	H2	H3	H4
0.35	0.24	0.24	0.21

produced in Treatment 1 than in either Treatments 2 or 3. The production of larger fruit as a result of fewer fruit per plant would, in Treatment 1, coincide with the abortion of some of the flower blossoms as previously noted. Due to the close resemblance of the number of fruits as well as

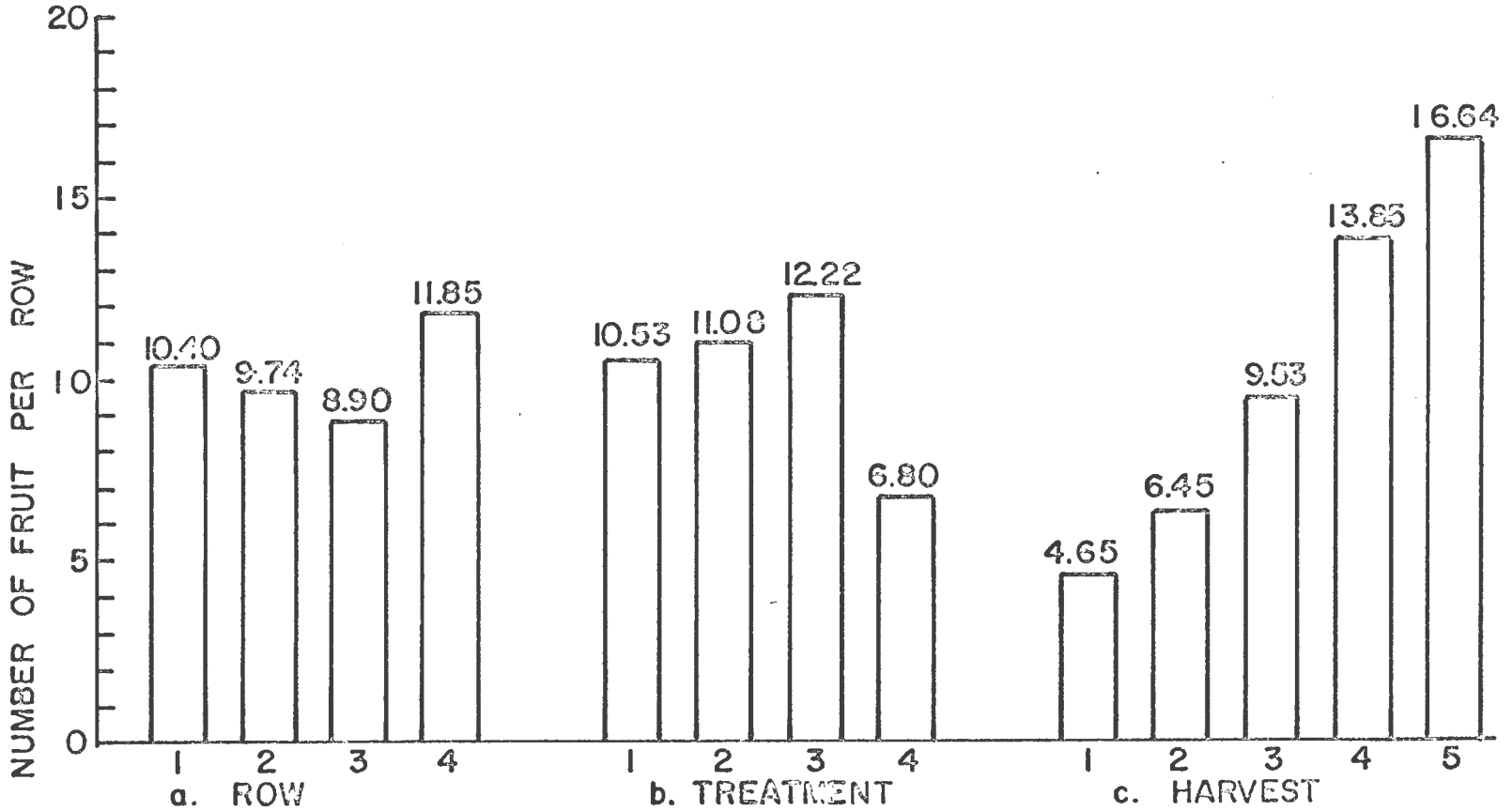

Figure 16. Means for number of tomato fruit per row

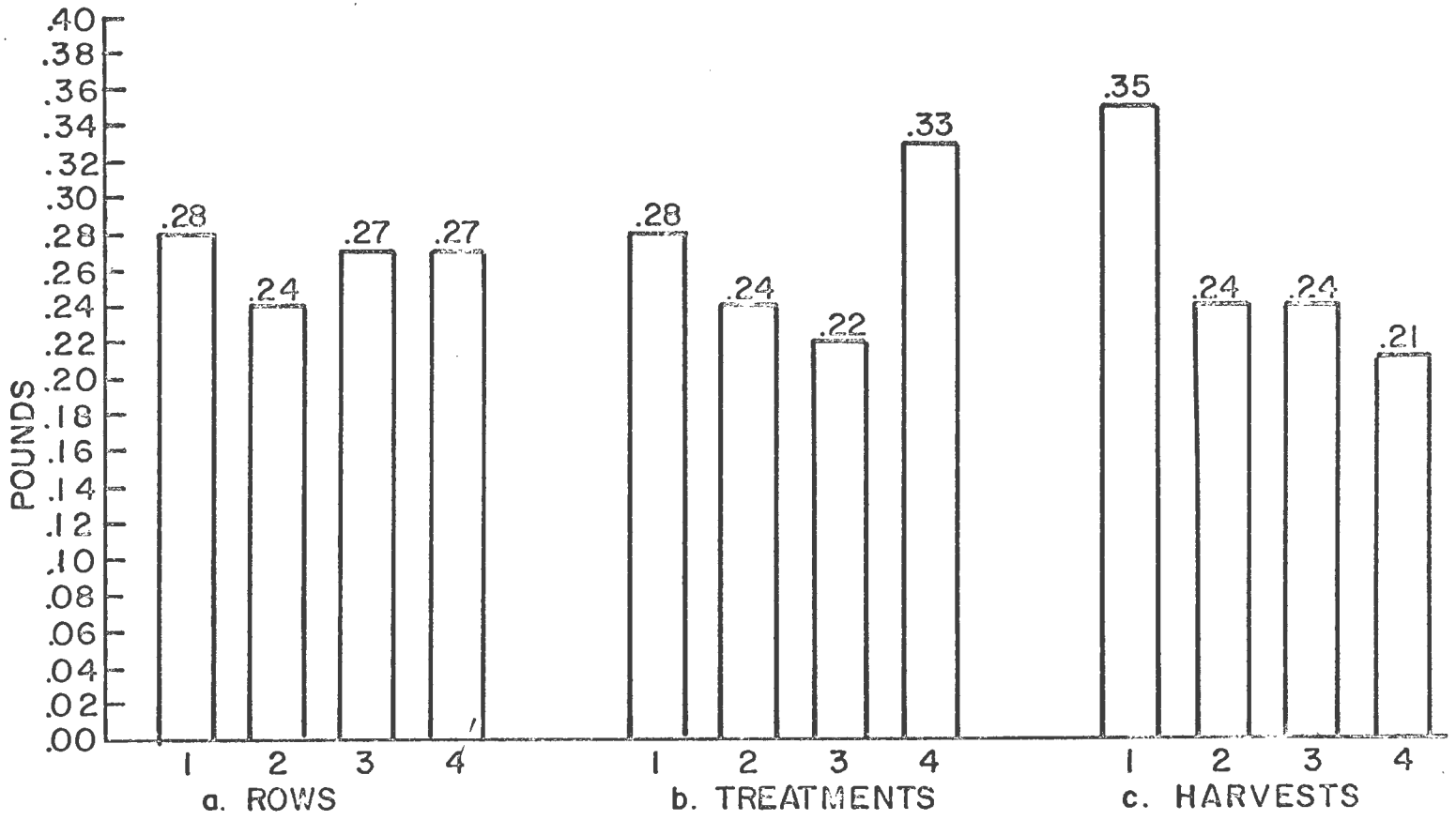

Figure 17. Means for average weight per tomato fruit

the size of fruit in Treatments 1, 2 and 3 when compared to Treatment 4, it is suggested that the environment was a major contributing factor in the variation between treatments by providing less than optimum growing conditions in Treatment 4 which consequently resulted in its decreased yield.

Number of fruit and size of fruit are inversely proportional to each other over all harvests as a result of larger and fewer fruits being produced in the later harvests (Figure 16c and 17c). The data indicate, however, that this relationship was not maintained by all treatments over all harvests (Figure 18 and 19). Treatment 1 initially produced the greatest number of fruit and larger fruit than Treatments 2 and 3; and in the final harvest Treatment 1 continued to produce the largest fruit but it also produced the lowest number of fruit. This again indicates an adverse effect on fruit set after several exposures. It is also evident that the greatest portion of large fruit produced in Treatment 4 was produced in the first harvest. This could possibly be in response to an earlier exposure to ambient 2,4-D. The larger number of fruit in Harvest 4 and the very low number in Harvest 5 of the fourth Treatment is due to the difference in harvest completion dates as previously described.

Several investigators (22, 39, 65, 67) have observed decreased yields with the use of sublethal concentrations of 2,4-D on several species. The use of spray drift and volatility in this study caused only a slight trend toward the reduction in yields of strawberries and greenbeans and caused a very slight increase in total yield of tomatoes.

The tomato yield data is in agreement with Guzman (36) who observed no significant effect on tomato yields from spray drift or volatility and also with Cole (13) who observed little effect on total yield but did

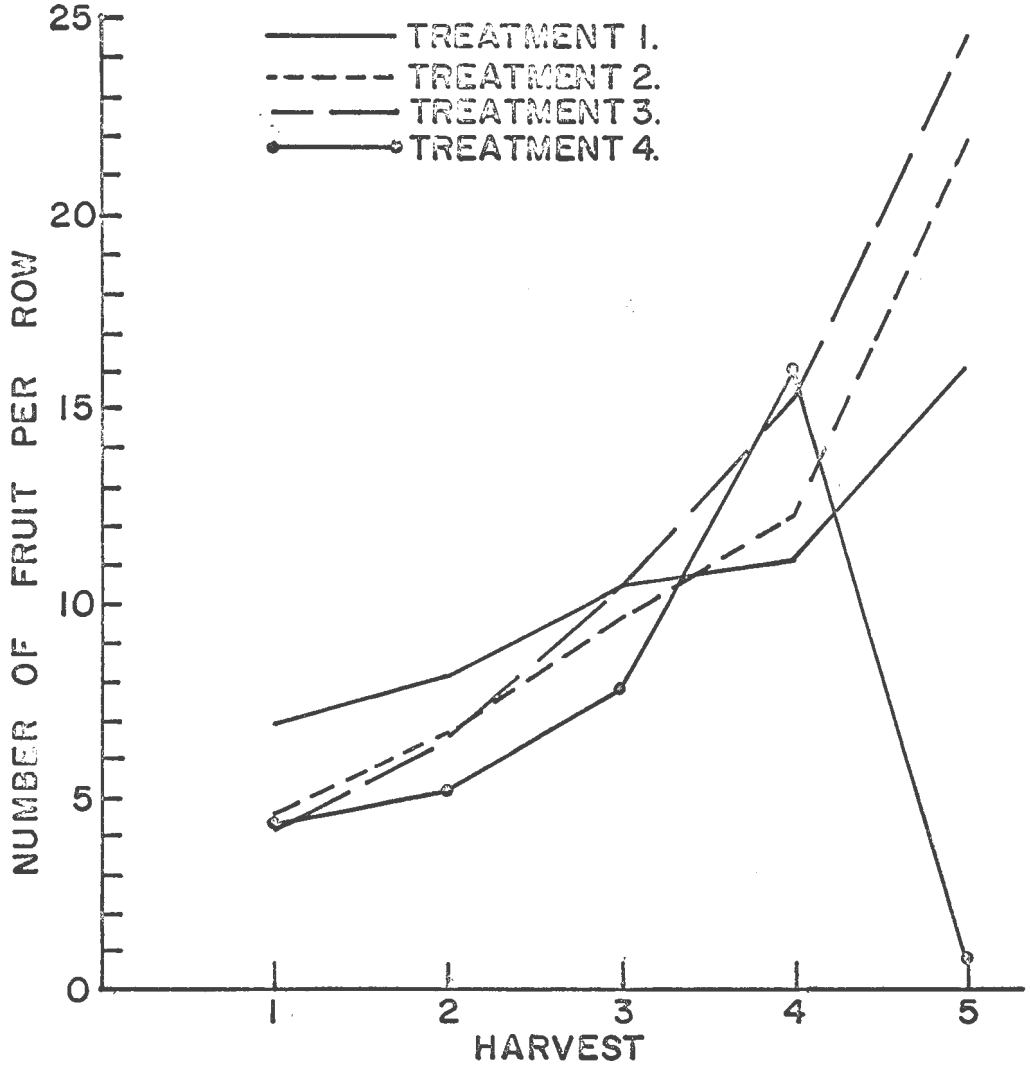

Figure 18. Treatment X harvest interaction for number of tomato fruits per row

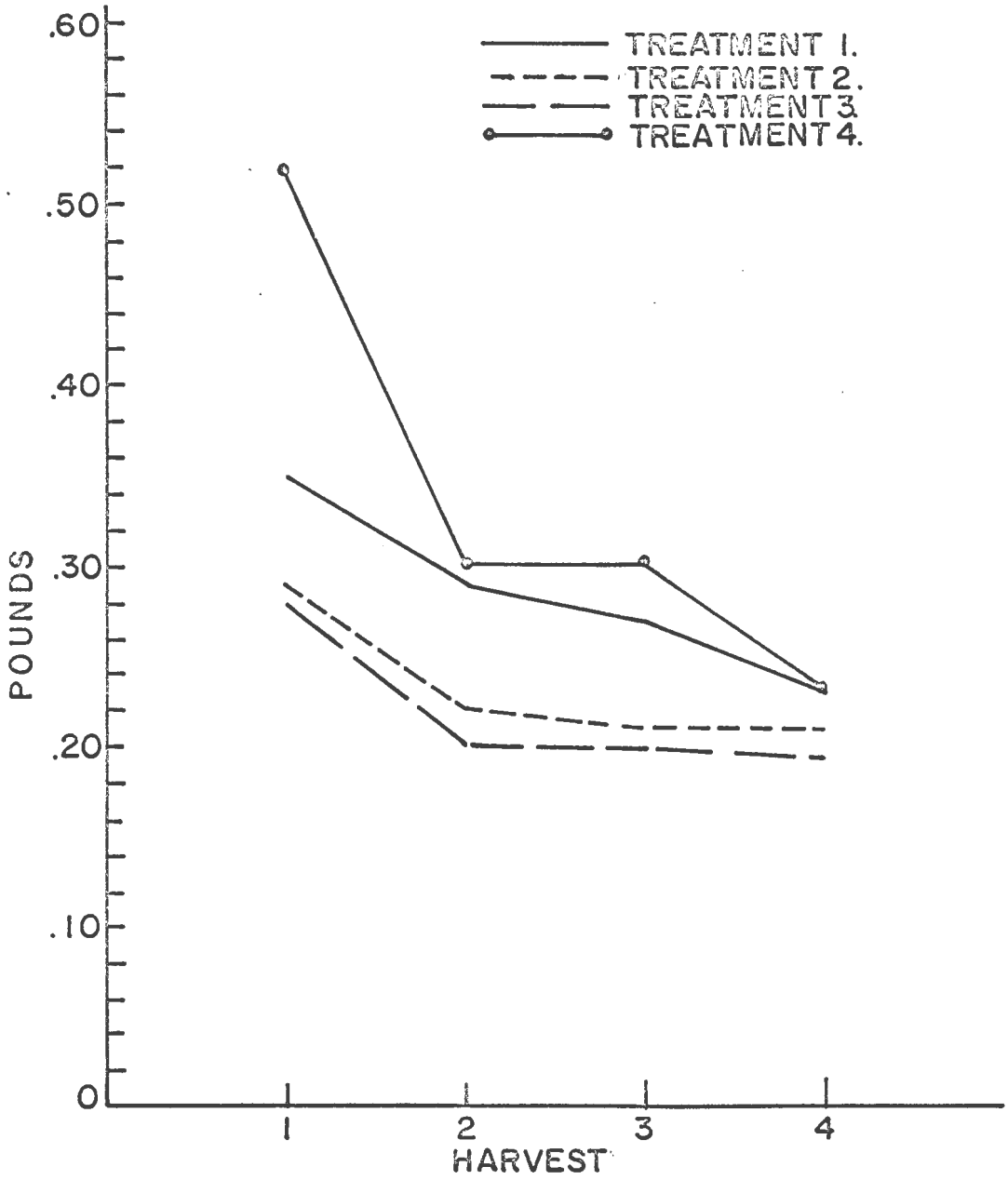

Figure 19. Treatment X harvest interaction for average weight per tomato fruit

report a slight increase in the average weight of grape clusters.

Parthenocarpic development

A highly significant difference in the average number of seeds per gram of fruit tissue was obtained between the three varieties used in this study (Table 10). With Campbell 1327, the distance from source did not appear to be a factor affecting this characteristic. Variety Manapal, previously reported as being somewhat tolerant to ambient 2,4-D[1] and also reported in this study as producing the highest yields, showed a significant reduction in the number of seeds per gram of fruit tissue. Variety Avalanche, the more sensitive variety and the variety that produced the lowest yield, showed a significantly greater number of seeds than any of the other varieties (Figure 20a). This relationship indicates that the effect of 2,4-D on fruit set and growth is independent or possibly negatively correlated with the effect of 2,4-D on parthenocarpic development.

The variation of parthenocarpy between treatments was significant at the 95% level where most of the significance was due to the small number of seeds per gram of fruit in Treatment 1. In a comparison of the data from the greenhouses, it is readily apparent that the release of 2,4-D into the atmosphere of Treatment 1 and the ambient 2,4-D in Treatment 3, significantly induced parthenocarpic development over that found in Treatment 2 (Figure 20b). It is also apparent that although volatile 2,4-D was present in Treatment 4, parthenocarpic development was greatly reduced.

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[1]Weigle, J. L. Iowa State University, Ames, Iowa. Data from field study on tomatoes. Private communication. 1966.

Table 10. Analysis of covariance for the average number of seeds per gram of tomato fruit tissue

Source of Variation	Degrees of Freedom	Sums of Squares	Mean Squares	F
Rows	3	7.23	2.41	14.18 **
Treatments	7	5.08	0.72	4.24 *
Error (a)	21	3.58	0.17	
Harvests	3	0.84	0.28	4.54 **
Treatments X Harvests	21	2.90	0.14	2.23 **
Error (b)	65	4.02	0.06	

Duncan's Multiple Range Tests

Rows: (99%)

R2	R3	R1	R4
1.37	1.04	1.03	0.66

Treatments: (95%)

T4	T2	T3	T1
1.20	1.13	0.92	0.58

Harvests: (99%)

H4	H3	H1	H2
1.15	1.02	0.99	0.92

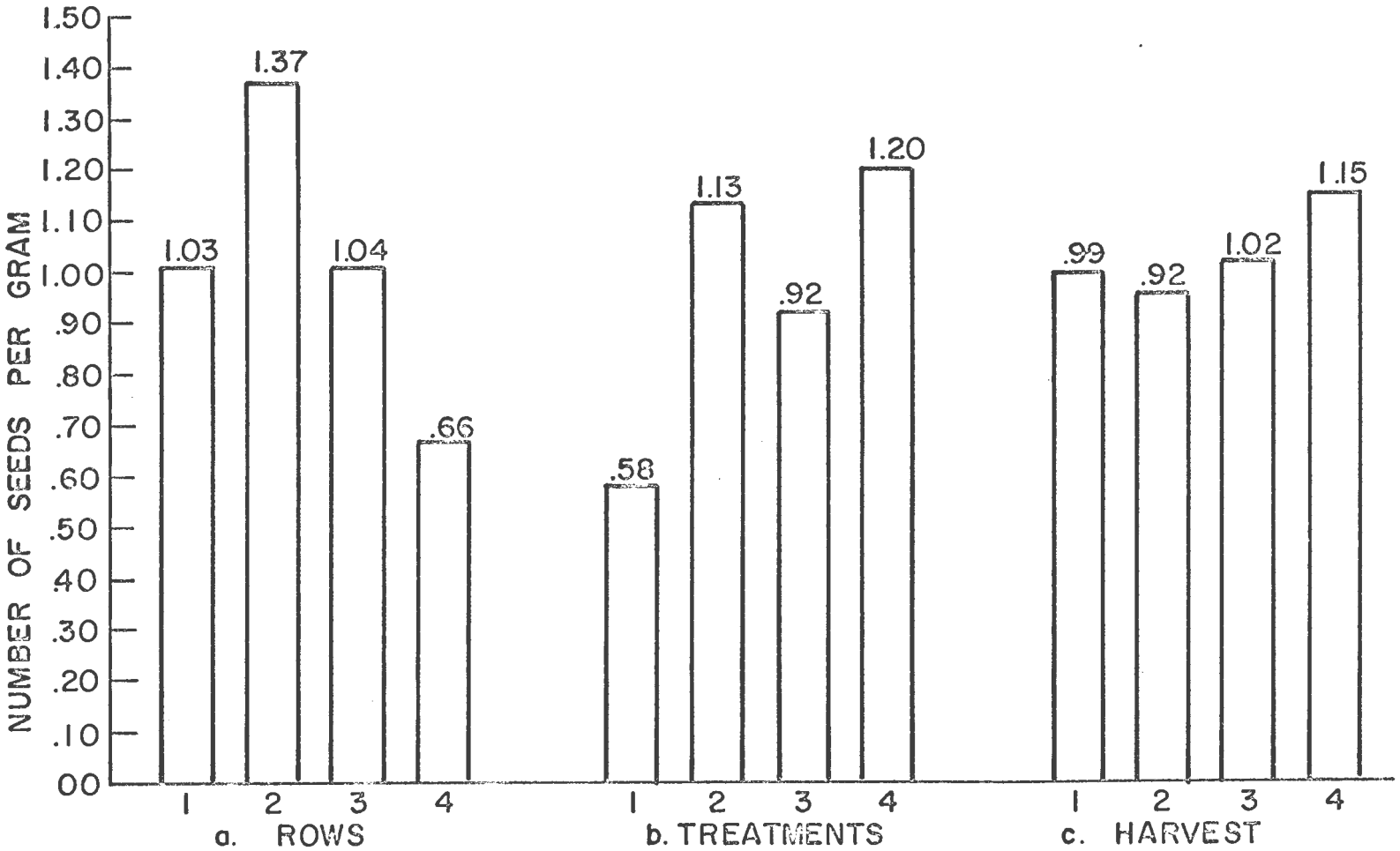

Figure 20. Means for average number of seeds per gram of tomato fruit tissue

Osborne and Went (63) induced parthenocarpy in tomatoes with high and low temperatures and high light intensities which are conditions in which pollination is poor. It is suggested, therefore, that the warmer air temperatures within the greenhouses may account for the greater degree of parthenocarpic development observed within the houses than in the outdoor plots.

The variation of seedless fruits with harvests was also significant at the 99% level with the degree of parthenocarpy increasing at first and then decreasing in the later harvests (Figure 20c). The increase could be due to the initial release of 2,4-D in Treatment 1 in late July and the decrease may probably be due to a decrease of volatile 2,4-D in the ambient atmospheres of Treatments 3 and 4 during the later part of the season. Gustafson (34) observed that seedless tomato fruits produced with B-naphthoxyacetic acid were larger than seeded fruits and that setting was also somewhat greater than with open pollination. In this study, a comparison of Treatments 1, 2 and 3 (Figures 17b and 20b) it is evident that larger fruits and more seedless fruits were produced in Treatment 1, however, fruit set was not increased accordingly (Figure 16b).

According to Bonner and Galston (7), the growth of fruit depends intimately on auxin and the source of this auxin is generally the developing seeds of the fruit. In the production of the many seedless fruit and fewer seeded fruits observed in Treatment 1, fruit development was, in most instances, brought about by influences other than pollination. According to several investigators (7, 33, 35, 47, 68) this influence or source of the required auxin is provided by the auxin-like properties of 2,4-D in the tissues of the fruit itself. The data from this experiment is also in

agreement with Zimmerman and Hitchcock (92) who have specifically shown that parthenocarpy is readily induced by several growth substances, such as 2,4-D in the vapor form. It has also been shown (47) that the absence of fertilization is not always necessary for parthenocarpic development as seedless fruits commonly result even though there has been some fertilization. In this case the auxin commonly brings about an abortion of the developing embryo which had been fertilized (47).

It is concluded that the parthenocarpic development observed in this study occurred as a result of the release of the butyl ester of 2,4-D in Treatment 1 and from ambient 2,4-D in Treatments 3 and 4. In Treatments 1, 2 and 3 parthenocarpic development was enhanced by the environmental effects created by the greenhouses. It is hypothesized that without these environmental effects, the degree of parthenocarpic development would have been less in Treatments 1, 2 and 3 but also that a greater number of seeded fruits would still have developed in Treatment 2 than in Treatments 1, 3 or 4.

Analysis of Vegetative Tissue of Tomato Plants

Percent dry matter

A significant difference in percent dry matter in the vegetative tissue of the tomato plants occurred among treatments (Table 11). The treatments exposed to either ambient 2,4-D (Treatments 3 and 4) or introduced 2,4-D (Treatment 1) had a higher percent dry matter than the cleaner atmosphere in Treatment 2, (Figure 21a). Treatment 4 had the highest amount of dry matter probably due to the environmental conditions causing less succulent growth in the outdoor plots. Regardless of the environmental

Table 11. Analysis of covariance for the percent dry weight of vegetative tomato tissue

Source of Variation	Degrees of Freedom	Sums of Squares	Mean Squares	F
Treatments	3	82.80	27.60	7.93 **
Variety	1	14.25	14.25	4.09 *
Error (a)	27	94.05	3.48	
Sampling Time	10	481.88	48.19	36.17 **
Treatments X Sampling Time	30	77.87	2.60	1.91 **
Error (b)	277	376.60	1.36	

Duncan's Multiple Range Tests
Treatments: (99%)

T4	T1	T3	T2
13.33	12.78	12.69	11.95

Sampling Time: (99%)

S11	S2	S8	S9	S7	S10	S1	S5	S3	S6	S4
14.86	14.02	13.54	13.05	12.91	12.81	12.56	12.12	11.72	11.55	10.43

effects, it is apparent that the presence of volatile 2,4-D caused an increase in dry weight probably as a result of increased differentiation of the tissue which was caused by the hardening of the plants in response to the 2,4-D exposures. This increased differentiation and hardening effect may also account for the greater tolerance of the plants to 2,4-D after several exposures as previously noted. Although several investigators have reported a depletion of food reserves (11, 40, 46, 51, 57, 73, 75) and a decrease in photosynthesis (2, 16, 38) and therefore a reduction in dry matter, it is noted that these decreases have occurred with the use of a higher concentration of 2,4-D (greater than 20 ppm) which also resulted in a considerable increase in the respiration rate. Wort (86) has reported that relatively high concentrations of 2,4-D have an adverse effect on the rate of photosynthesis but that lower concentrations applied at the correct time may have the opposite effect resulting in a greater production of dry matter. Brown (11) observed a 34% decrease in the total amount of water absorbed and transpired by sprayed plants and that the rate of accumulation of water in the leaves was depressed while in the stem tissue it was accelerated. Freiberg and Clark (30) reported that within 23 hours after an exposure to 4 ppm of 2,4-D the percent dry matter of the leaves was significantly decreased, but that after several days they showed a much higher percent of dry matter. These observations substantiate the results of this experiment and also contribute to the explanation of significance in the treatment sampling time interaction. It is suggested, however, that the different rates of dry matter accumulation among treatments over the different sampling times and the large amount of variation with sampling time were the main factors in this interaction. It is

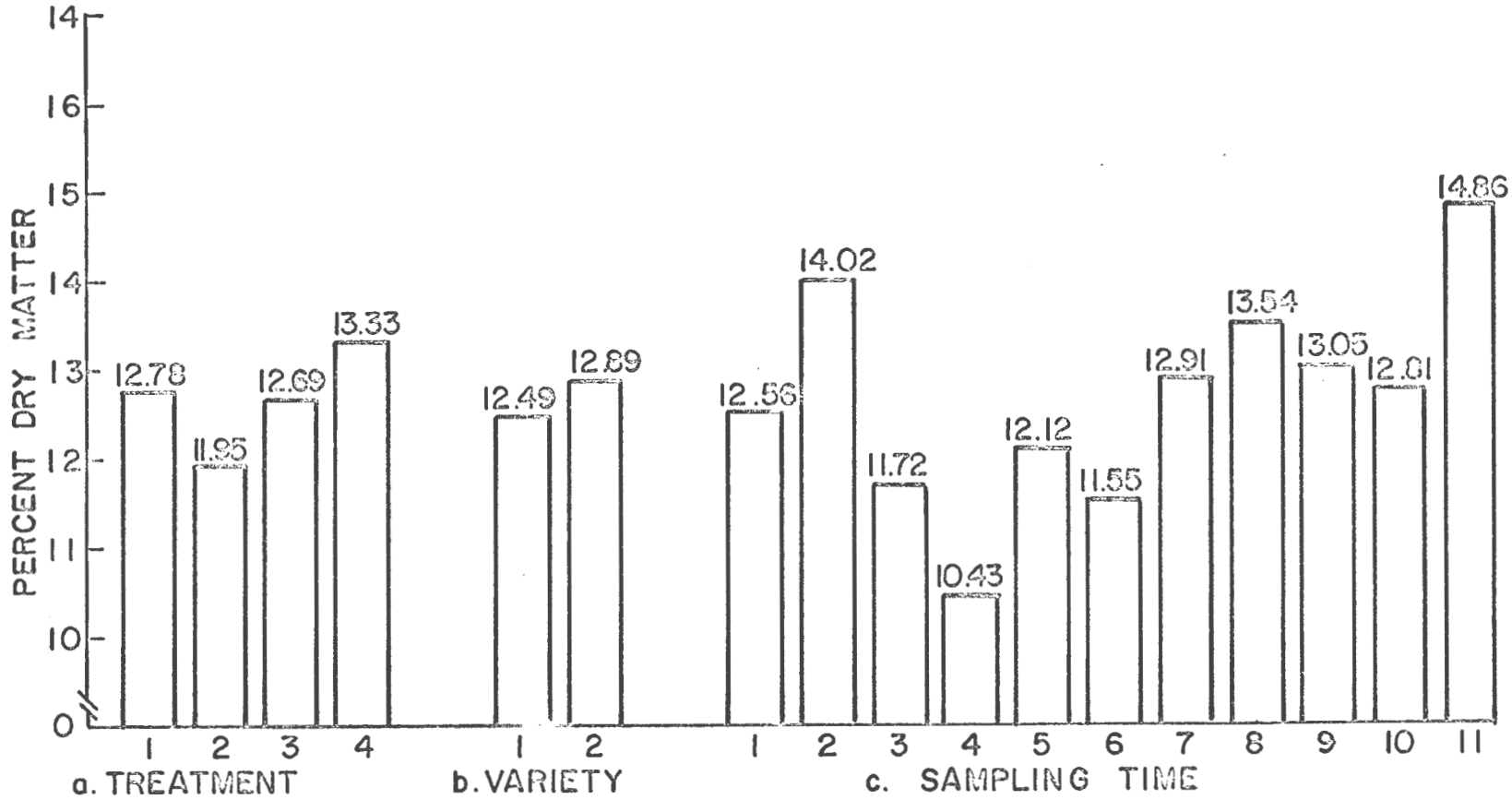

Figure 21. Percent dry matter in tomato vegetative tissue

apparent from the literature that the effect of 2,4-D on the percent dry matter in plant tissue is strongly dependent on the concentration used. It is therefore probable that the concentration of 2,4-D used in this study was responsible for an increase in dry matter of tomato plants through possibly a slight increase in photosynthesis, an adverse effect on the water relations of the plants and also an increase in differentiation as a result of the stress conditions created by the 2,4-D injury.

The lower percent of dry matter in the Campbell 1327 variety than the Avalanche variety (Figure 21b) is probably due to the inherent characteristics of the variety itself. The significant changes in percent dry matter over sampling time (Figure 21c) may probably be partly due to the various effects of 2,4-D on the water relations of the plants. It is suggested, however, that the greatest part of this variation exists due to differences in air temperature within the greenhouses at the time of sampling. A correlation was evident between these two variables.

2,4-D content

A significantly different content of 2,4-D in the vegetative tissue of the tomato plants was observed between treatments and also between sampling times (Table 12). No difference was obtained between varieties. It is apparent that the plants in Treatments 1 and 4 contained a significantly higher amount of 2,4-D than in Treatment 2 (Figure 22a). Although Treatment 3 was exposed to ambient 2,4-D as in Treatment 4, the plant tissue did not contain as much 2,4-D even though it did exhibit considerable injury, usually greater than that found outside. This is believed due to two factors: 1) it is suggested that as the ambient air is pulled

Table 12. Analysis of covariance for the ratio of 2,4-D content in vegetative tomato tissue

Source of Variation	Degrees of Freedom	Sums of Squares	Mean Squares	F
Treatments	3	0.01107	0.00369	10.25 **
Variety	1	0.00018	0.00018	0.50
Error (a)	27	0.00969	0.0036	
Sampling Time	10	0.05419	0.00542	19.36 **
Treatments X Sampling Time	30	0.01275	0.00042	1.50
Error (b)	277	0.07735	0.00028	

Duncan's Multiple Range Tests
Treatments: (99%)

	T1	T4	T3	T2
Ratio	0.0458	0.0436	0.0376	0.0314
ppb	48.0	45.6	39.0	32.4

Sampling Time: (99%)

S8	S6	s9	S3	S11	S7	S10	S4	S2	S5	S1
Ratio										
0.0650	0.0513	0.0468	0.0433	0.0416	0.0415	0.0412	0.0364	0.0242	0.0239	0.0208
ppb										
69.5	54.1	49.1	45.2	43.4	43.3	43.0	37.8	24.8	24.5	21.2

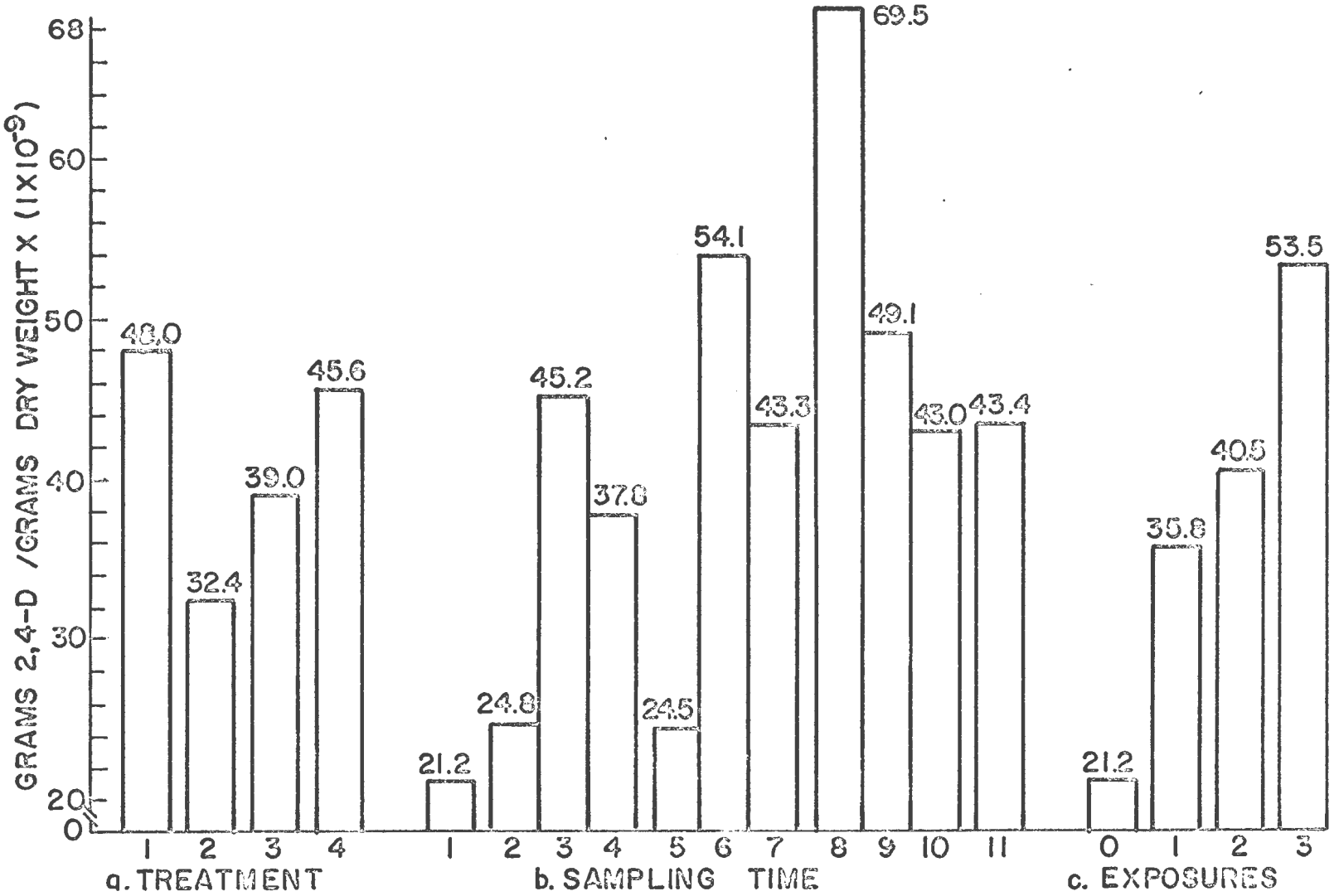

Figure 22. Content of 2,4-D in ppb of dry matter in vegetative tissue of tomato plants

through the wet excelsior pads, the water flowing over these pads could have adsorbed or trapped a small portion of any 2,4-D that was present. This would therefore slightly reduce the actual amount of 2,4-D released into the house atmosphere; 2) in spite of the decreased amount of 2,4-D within the houses, the environmental conditions again resulted in more rapid and succulent growth which subsequently enhanced any injurious effects of the 2,4-D. The presence of the 2,4-D found in Treatment 2 substantiates the plant injury observed in this treatment as previously noted.

Without considering the variation in absorption of 2,4-D by the plants and also the variation in degradation of 2,4-D within the plants of Treatments 1 and 4, the data indicate that the two treatments received similar exposure of 2,4-D although Treatment 1 was shorter and more concentrated and Treatment 4 was assumed to be less concentrated and continuous. Considering then, the degree of injury observed in Treatments 1 and 4 and also the quantity of 2,4-D found in the plant tissue of the two treatments, a rough estimate can be calculated as to the approximate concentration of 2,4-D in the ambient atmosphere. Assuming an average continuous concentration level of ambient 2,4-D compared to the known concentration level of 1.18 $\mu gm/m^3$ for six hours in Treatment 1, it is estimated that the ambient level of 2,4-D would be only 3 to 4 percent as great as Treatment 1 or approximately 0.04-0.05 $\mu gm/m^3$. This would be slightly less than the average quantity of 2,4-D found in the ambient atmosphere by Adams et al. (1) in 1964.

Figure 22b demonstrates the response obtained as to the quantity of 2,4-D present in the plant tissue at each harvest date. Each bar represents a single sampling date and the three groups of three bars each

represent the three samples taken after three separate exposures. Sampling Time 1 occurred prior to any exposures and Sampling Time 11 occurred after ten exposures to 2,4-D. Figure 22c represents the mean 2,4-D content of the tissues over all observations from each exposure. It has been reported (4, 18, 47) that most of the absorption of 2,4-D by plants occurs within 30 minutes to 10 hours from the time of treatment depending on temperature, concentration, plant species and formulation of the spray. The results of this experiment indicate that with the use of 2,4-D in the vapor form, absorption was somewhat retarded, as the greatest amount was absorbed during the first 24 hours with most of this absorbed after six hours from the time of each exposure. The increased amount of 2,4-D present during this time could also be a result of increased translocation of 2,4-D along with carbohydrates to the actively growing portion of the plants, as it was this part of the plant that was analyzed. Ninety-six hours after each exposure, the 2,4-D content had decreased, probably as a result of a degradation of the compound to certain metabolites or a loss of part of the chemical in the form of CO_2 as reported by Slife et al. (69). However, as indicated by Figure 22c, this rate of metabolism of the 2,4-D molecule was exceeded by the rate of absorption and translocation of additional 2,4-D into the apical portion of the plant resulting in an accumulation of the compound in the vacuoles of active parenchyma cells which according to Crafts (16) represents a type of storage. Because of this accumulation, a greater quantity of 2,4-D is present within the tissue than the quantity released to the atmosphere within Treatment 1. As a result of this experiment it is concluded that 2,4-D is absorbed by the entire plant, translocated to regions of active growth and accumulated there to toxic

concentrations which result in injury to the plant tissue. As reported previously, this injury occurred within 24 hours after the second exposure. The lower 2,4-D content observed in Sampling Time 11 is probably due to a decrease in ambient 2,4-D throughout the summer and also to a diversion in translocation of 2,4-D from the apical portions of the plant to the active sinks created by the flowers and fruit.

Considering the effects of the environmental conditions and other physical characteristics in addition to the growth condition of the plants involved in this study, the degree of injury appears to coincide quite well with the differences in the amount of 2,4-D exposure as well as the quantity of 2,4-D found within the plant tissue. In general, with exception of the problems previously cited concerning Treatment 3, the greatest injury occurred in the plants exposed to slightly higher concentrations and also where the 2,4-D content of the tissue was the highest. This is specifically emphasized by the observations obtained in Treatment 1 where 1.18 $\mu gm/m^3$ of the butyl ester of 2,4-D was released and caused severe injury, and by Treatment 2 where most of the 2,4-D was filtered from the ambient atmosphere and therefore caused very little injury.

SUMMARY

Visual symptoms of 2,4-D injury on strawberry, raspberry, tomato and greenbean plants and its effect on yield and size of fruit was investigated using ambient 2,4-D and 1 ppb of the butyl ester of 2,4-D in a vaporized form. The effects of 2,4-D on parthenocarpic development of tomato fruit and on dry matter content in the vegetative tissue of tomato plants were also studied. A determination was made on the content of 2,4-D within vegetative tissue of tomato plants.

1) Symptoms of severe injury occurred on the grapes and tomatoes from both ambient and atomized sources of 2,4-D. Injury was manifested by mottling, deformation of leaves and vein clearing in the grapes and by epinasty of terminal shoots with longitudinal curling of leaves and subsequent rolling of the midrib on tomatoes. No injury was apparent on either strawberry or raspberry plants for the duration of the season.

2) No significant difference in yield of strawberries was observed between treatments, however, a slight reduction in yield was noted in the ambient atmosphere and the atmosphere in which 2,4-D was released. A slight decrease in size of fruit and a slight deformation of fruit also existed in these three treatments.

3) The difference obtained in greenbean yields were attributed to environmental influences and therefore the effect of 2,4-D was considered neglibible.

4) The Manapal variety of tomatoes appeared to be the most tolerant to 2,4-D in that it produced the largest yield. Varieties Avalanche and Campbell 1327 were similar in yield depending on the distance from source

of 2,4-D release in which the higher yield was produced furthest from the source. Variety Manapal also produced the greatest number of fruit and Campbell 1327, closest to the source of 2,4-D, produced the least fruit. Campbell 1327 produced the largest fruit.

5) With the exception of the outdoor plots (Treatment 4), a trend toward an increase in tomato yields existed in the 2,4-D contaminated atmospheres. The plants exposed to 1 ppb 2,4-D (Treatment 1) produced higher earlier yields, but lower later yields. Size of fruit and number of fruit varied inversely with each other with the largest and fewest fruit produced in Treatments 1 and 4. Significantly larger tomatoes were produced in the atmosphere contaminated with the butyl ester of 2,4-D than in the clean atmosphere.

6) Variety Manapal exhibited the greatest amount of parthenocarpic development and Avalanche showed the least. Variety Campbell 1327 was intermediate with distance from source not being a factor. The greatest amount of parthenocarpy occurred in Treatment 1 and the least in Treatment 4, however, this phenomenon was greatly enhanced by the environment created within the plastic greenhouses. A significant increase in parthenocarpic development did, never-the-less, occur as a result of exposure to the butyl ester of 2,4-D.

7) The presence of volatile 2,4-D in Treatments 1, 3 and 4 resulted in a significant increase of the percent dry matter in the vegetative tissue of tomato plants.

8) A significantly higher quantity of 2,4-D was detected in the tissue of tomato plants exposed to the butyl ester of 2,4-D. The plants exposed to ambient atmosphere also contained a higher content of 2,4-D than

Treatment 2. The 2,4-D content of the tissue increased during the first 24 hours following exposure and then decreased slightly until the next exposure. This decrease, however, was exceeded by the accumulation of 2,4-D within the tissue resulting in an accumulative increased 2,4-D content with each additional exposure.

9) The effects of environmental conditions upon the results are noted and discussed.

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